

Université de Montréal

**Contribution of tachykinin and kinin receptors in central autonomic control
of blood pressure and behavioural activity in hypertensive rats**

par

Helaine De Brito Pereira

Département de physiologie

Faculté de Médecine

Thèse présentée à la Faculté des études supérieures et postdoctorales

en vue de l'obtention du grade de

Philosophia Doctor (Ph.D.)

en Physiologie

Mai, 2010

Université de Montréal
Faculté des études supérieures et postdoctorales

Cette thèse intitulée:

**Contribution of tachykinin and kinin receptors in central autonomic control
of blood pressure and behavioural activity in hypertensive rats**

Présentée par :

Helaine De Brito Pereira

a été évaluée par un jury composé des personnes suivantes :

Dr. Jean-Louis Schwartz, président-rapporteur

Dr. Réjean Couture, directeur de recherche

Dr. Madhu B. Anand-Srivastava, membre du jury

Dr. Pedro D'Orléans-Juste, examinateur externe

Dr. Hélène Girouard, représentant de la FESP

I want officially thank God, for giving me the patience, health, strength, and intelligence to do my doctoral studies.

I am thankful for the kindest, most understanding and loving husband in the world. I cannot even put into words the kind of man he is. I am blessed that God brought him into my life and thankful for the almost six years that we have shared so far.

I would like to thank my siblings Renato and Karina, and my brother-in-law Fabio for treasured memories and loving support throughout my education and graduation.

Aos meus pais,

Que me deram a vida e me ensinaram a vivê-la com dignidade,

não bastaria um obrigado.

Que iluminaram os caminhos obscuros com afeto e dedicação para que os
trilhássemos sem medo e cheios de esperanças,

não bastaria um muito obrigado.

Que se doaram inteiros e renunciaram aos seus sonhos, para que, muitas vezes,
pudésse realizar os meus. Pela longa espera e compreensão durante minha longa
ausência, não bastaria um muitíssimo obrigado.

A vocês, pai e mãe por natureza, por opção e amor, não bastaria dizer, que não
tenho palavras para agradecer tudo isso. Mas é o que me acontece agora, quando
procuro arduamente uma forma verbal de exprimir uma emoção incomparável.

Uma emoção que jamais seria traduzida por palavras.

Amo vocês

Acknowledgements

I want first to thank my research director, Dr. Rejean Couture, for welcoming me in Canada and in his laboratory. During more than a decade of knowing him, he has helped me to see life and science in their full depth, and taught me how to appreciate the good scientific work that helps other researchers to build on it.

I warmly thank my laboratory friends and colleagues, Sébastien Talbot, Dr. Brice Ongali, Dr. Frank Cloutier, Jenny Pena-Dias, Mahmud Ismael, Emna Chahmi, Nejla Tidjane, Dr. Karim Lahjouji, and Jacques Sénécal.

Thank you to all members of the Physiology department, and especially to Johanne Payette and Marjolaine Homier, for their help and support.

I would like to say thank you to Paula Jansen Santana and Claude Gauthier for the graphic and artistic work on the figures.

In addition, I thank my Brazilian friends who encouraged, supported me and have always been present in hard times, Janaina Suckow, Simone Carrer, Angela Piovesan, Billa Furlanetto, THANK YOU and I love you girls!

Finally, I thank the Quebec Society of Hypertension (SQHA) and the Faculty of Graduate Studies (FESP), which have offered me travel grants and awards of excellence, respectively. The financial support of this work was provided by research grants from the Canadian Institutes of Health Research (CIHR) to Dr. Réjean Couture and National Council of Technological and Scientific Development (CNPq) (proc. 201428/2003-2) Brazil to H. De Brito Pereira.

Abstract

This work aims at studying the role of tachykinin NK-3 receptor (R) and kinin B1R in central autonomic regulation of blood pressure (BP) and to determine whether the B1R is overexpressed and functional in rat models of hypertension by measuring the effect of a B1R agonist on behavioural activity. Assumptions: (1) NK-3R located in the ventral tegmental area (VTA) modulates the mesolimbic dopaminergic system and has a tonic activity in hypertension; (2) B1R is overexpressed in the brain of hypertensive rats and has a tonic activity, which contributes to hypertension via a dopamine mechanism; (3) the inhibition of NK-3R and B1R with selective antagonists, reduces central dopaminergic hyperactivity and reverses hypertension. A model of genetic hypertension and a model of experimental hypertension were used: spontaneously hypertensive rats (SHR, 16 weeks) and Wistar-Kyoto (WKY) rats infused for 14 days with angiotensin II (Ang II) (200 ng / kg / min, subcutaneous (s.c.) with Alzet mini pump). The age-matched untreated WKY rats served as common controls. In the first study (article # 1), the cardiovascular response in SHR was evaluated following intracerebroventricular (i.c.v.) and/or intra-VTA injection of an agonist (senktide) and antagonists (SB222200 and R-820) of NK-3R. These responses have also been characterized using selective dopamine antagonists DA-D1R (SCH23390), DA-D2R (raclopride) or non-selective dopamine DA-D2R (haloperidol). Also the VTA has been destroyed by ibotenic acid. The pressor response induced by senktide and the anti-hypertensive response induced by SB222200 or R-820 were more pronounced by intra-VTA. These responses were prevented by pre-treatment with raclopride and haloperidol. The lesion of the VTA has prevented the pressor response relayed by senktide (i.c.v.) and the anti-hypertensive effect of R-820 (i.c.v.). In addition, SB222200 (intra-VTA) prevented the pressor response of senktide (i.c.v.) and conversely, senktide (i.c.v.) prevented the antihypertensive effect of SB222200 (intra-VTA). The second study (article # 2) showed that the B1R antagonist (SSR240612)

administered by gavage or i.c.v. reverses hypertension in both models. This anti-hypertensive effect was prevented by raclopride and haloperidol. In contrast, the two B1R antagonists (R-715 and R-954) injected s.c., which do not cross the blood-brain barrier reduced weakly blood pressure in hypertensive rats. In the third study (article # 3), the i.c.v. injection of a selective kinin B1R agonist Sar[DPhe⁸][des-Arg⁹]BK caused behavioural responses in SHR and Ang II-treated rats and had no effect in control WKY rats. The responses elicited by B1R agonist were blocked by an antagonist of NK-1 (RP67580), an antagonist of NMDA glutamate receptor (DL-AP5), an inhibitor of nitric oxide synthase (NOS) (L-NNA) as well as raclopride and SCH23390. The responses were modestly affected by the inhibitor of inducible NOS (iNOS). The B1R mRNA (measured by RT-PCR) was significantly increased in the hypothalamus, the VTA and the nucleus accumbens of hypertensive animals (SHR and treated with Ang II) compared with control rats.

These neuropharmacological studies suggest that: (1) the NK-3R from the VTA is involved in the maintenance of hypertension in SHR by increasing DA transmission in the midbrain; (2) the B1R in SHR and Ang II-treated rats contributes to hypertension via a central mechanism involving DA-D2R; (3) the central B1R increases locomotor activity and nocifensive behaviours via the release of substance P (NK-1), DA and nitric oxide in both rat models of hypertension.

Thus, the brain tachykinin NK-3R and kinin B1R represent potential therapeutic targets for the treatment of hypertension. The modulation of the mesolimbic/mesocortical dopaminergic pathway by these receptors suggests their involvement in other physiological functions (pleasure, motor activity, coordination of the response to stress) and pathophysiology (anxiety, depression).

Keywords: Tachykinin NK-3R, kinin B1R, dopamine, ventral tegmental area, hypertension, behavior.

Résumé

Ce travail vise à étudier le rôle du récepteur NK-3 des tachykinines (NK-3R) et du récepteur B1 des kinines (B1R) dans la régulation autonome centrale de la pression artérielle et de déterminer si le B1R est surexprimé et fonctionnel chez le rat hypertendu en mesurant l'effet d'antagoniste B1R sur l'activité comportementale. Hypothèses: (1) le NK-3R localisé dans l'aire tegmentale ventrale (VTA) module l'activité dopaminergique du système mésolimbique et possède une activité tonique dans l'hypertension; (2) le B1R est surexprimé dans le cerveau du rat hypertendu et possède une activité tonique qui contribue à l'hypertension via un mécanisme dopaminergique; (3) l'inhibition des NK-3R et B1R avec des antagonistes sélectifs réduit l'hyperactivité dopaminergique centrale et renverse l'hypertension. Un modèle d'hypertension génétique et un modèle d'hypertension expérimentale ont été utilisés: le rat spontanément hypertendu (SHR, 16 sem) et le rat Wistar Kyoto (WKY) infusé pendant 14 jours avec l'angiotensine II (Ang II) (200 ng/kg/min, s.c. avec mini pompe Alzet). Le rat WKY non traité du même âge a servi de témoin commun. Dans la première étude (article # 1), la réponse cardiovasculaire des SHR a été évaluée à la suite de l'injection i.c.v. et/ou intra-VTA d'un agoniste (senktide) et d'antagonistes (SB222200 et R-820) du NK-3R. Ces réponses ont aussi été caractérisées en utilisant des antagonistes sélectifs des récepteurs DA-D1R (SCH23390), DA-D2R (raclopride) ou non-sélectif DA-D2R (halopéridol). Aussi le VTA a été détruit par l'acide iboténique. La réponse pressive induite par senktide et la réponse anti-hypertensive induite par SB222200 ou R-820 étaient plus marquées par la voie intra-VTA. Ces réponses ont été prévenues par un pré-traitement avec le raclopride et l'halopéridol. La lésion du VTA a prévenu la réponse pressive relayée par le senktide (i.c.v.) ainsi que l'effet anti-hypertenseur du R-820 (i.c.v.). De plus, le SB222200 (intra-VTA) a prévenu la réponse pressive du senktide (i.c.v.) et inversement, le senktide (i.c.v.) a prévenu l'effet anti-hypertenseur du SB222200 (intra-VTA). La deuxième étude

(article # 2) a montré que l'antagoniste du B1R (SSR240612) administré par gavage ou i.c.v. renverse l'hypertension artérielle dans les deux modèles. Cet effet dépresseur a été prévenu par le raclopride ainsi que l'halopéridol. Par contre, le traitement avec deux antagonistes du B1R (R-715 et R-954) qui ne traversent pas la barrière hémato-encéphalique a réduit faiblement la pression artérielle chez les rats hypertendus. Dans la troisième étude (article # 3), l'injection i.c.v. d'un agoniste sélectif du B1R, le Sar[DPhe⁸][des-Arg⁹]BK a causé des réponses comportementales typiques chez le SHR et le rat traité à l'Ang II mais il n'a pas eu d'effet chez le rat témoin WKY. Les réponses induites par l'agoniste B1R ont été bloquées par un antagoniste du récepteur NK-1(RP67580), un antagoniste du récepteur NMDA du glutamate (DL-AP5), un inhibiteur des synthétases du monoxyde d'azote (NOS) (L-NNA) ainsi qu'avec le raclopride et le SCH23390. Les réponses ont été modestement influencées par l'inhibiteur de la NOS inductible (iNOS). L'ARNm du B1R (mesuré par RT-PCR) était significativement augmenté dans l'hypothalamus, le VTA et le noyau accumbens des animaux hypertendus (SHR et traités à l'Ang II) comparativement aux rats témoins.

Ces études neuropharmacologiques suggèrent : (1) que le NK-3R du VTA est impliqué dans le maintien de l'hypertension chez le SHR en augmentant la transmission DA au niveau du mésencéphale. (2) Le B1R chez le SHR et les rats traités à l'Ang II contribue à l'hypertension artérielle via un mécanisme central impliquant le DA-D2R. (3) le B1R central augmente l'activité locomotrice et les comportements défensifs, via la relâche de substance P (NK-1), de DA et de NO dans un modèle d'hypertension génétique et expérimental chez le rat.

Ainsi, les récepteurs cérébraux NK-3 des tachykinines et B1 des kinines représentent des cibles thérapeutiques potentielles pour le traitement de l'hypertension artérielle. La modulation de la voie dopaminergique mésolimbique/mésocorticale par ces récepteurs suggère une participation

dans d'autres fonctions physiologiques (plaisir, activité motrice, coordination de la réponse au stress) et en pathophysiologie (anxiété, dépression).

Mots clés : Tachykinine NK-3R, kinine B1R, dopamine, aire tegmentale ventrale, hypertension, comportement.

Index

Dedication	iii
Acknowledgements	v
Abstract	vi
Résumé	viii
Index	xi
List of Figures	xv
List of Tables.....	xxiii
List of Abbreviations	xxiv
Chapter I	1
1. General introduction.....	2
1.0.1 The autonomic nervous system: A strategic role in blood pressure regulation	2
1.0.2 Hypertension	3
1.0.3 The tachykinins and their receptors: regulation of central cardiovascular function in hypertension	3
1.0.4 The kinins and their receptors: regulation of central cardiovascular function in hypertension	6
1.1 Tachykinins	7
1.1.2 Tachykinins: biosynthesis and distribution	11
1.2 The NKA and NKB.....	16
1.3 Tachykinin receptors.....	18
1.4 Tachykinins: agonists and antagonists.....	19
1.4.1 NK-1R.....	19
1.4.2 NK-2R.....	26
1.4.3 NK-3R.....	29
1.5 Molecular biology of tachykinin receptors	34
1.6 Intracellular signaling pathways	35
1.7 Tachykinins in cardiovascular regulation	37
1.8 Tachykinins in spinal nociception	40
1.9 Tachykinins in behavioural activity	40

2. The ventral tegmental area	42
2.1 Location in the CNS	42
2.2 Roles of the VTA.....	42
2.2.1 Neuronal efferent and afferent of the VTA.....	44
2.2.1.1 Major efferents	44
2.2.1.2 Major afferents	47
2.3 Main receptors and neurotransmitters found in the VTA.....	47
2.4 Location and function of tachykinins in the VTA	49
3. Centrally acting anti-hypertensive agents.....	51
3.1 Clonidine	53
3.2 Guanabenz	54
3.3 Moxonidine	54
3.4 Methyldopa	54
4. Dopamine	55
4.1 Dopamine receptors	56
4.2 Dopamine: molecular signaling pathways	60
4.3 Dopamine in cardiovascular regulation	63
5. The kallikrein-kinin system	65
5.1 Brief history	65
5.2 Kininogens	68
5.3 Formation of kinins by kallikreins	69
5.4 Kallikreins distribution.....	71
5.5 Kininases	72
6. Kinin receptors	75
6.1 B1 receptors	75
6.1.2 Mechanism of the B1R induction	78
6.1.3 B1R agonists.....	80
6.1.4 B1R antagonists	81
6.2 B2 receptors	85
6.2.1 B2R agonists.....	85
6.2.2 B2R antagonists	86
6.2.3 B2R expression	91

6.3 Cloning of kinin receptors	92
6.3.1 B1R cloning	92
6.3.2 B2R cloning	93
6.3.3 Intracellular signaling pathways	93
7. Cardiovascular effects of kinins	97
8. Kinins receptors in the CNS	100
9. Kinins in the CNS	103
10. Action of kinins on body temperature	103
Chapter II	105
11. Objectives	106
11.1. First objective	106
11.2. Second objective	106
11.3 Third objective	107
Chapter III	108
12. Articles	109
12.1 Blockade of tachykinin NK-3 receptor reverses hypertension through a dopaminergic mechanism in the ventral tegmental area of spontaneously hypertensive rats	110
12.2 Contribution of the central dopaminergic system in the anti- hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension	159
12.3 Mechanism underlying behavioural activity induced by activation of brain kinin B ₁ receptor in hypertensive rats	187
Chapter IV	217
13. General discussion	218
13.1 Central tachykinin NK-3R in hypertension	218
13.2 Central kinin B1 receptors in hypertension and behavioural activity	223
13.3 Other drugs with central effects	230
14. Perspectives	232
15. General conclusion	237
16. References	238

17. Appendix	301
---------------------------	-----

List of Figures

Chapter I

Figure 1. Schematic representation of the TAC1, TAC3 and TAC4 genes expression and their mRNA messengers	12
Figure 2. Distribution of immunoreactive-SP in the rat hypothalamus	14
Figure 3. Schematic representation of the rat and human tachykinin NK-1R	20
Figure 4. Location of the VTA in the rat brain.....	43
Figure 5. Schematic midsagittal section of a rat brain, showing the locations of the most important groups of dopaminergic neurons and the distribution of their axons and terminal buttons	46
Figure 6. Schematic representation of the DA receptors in the human brain.	59
Figure 7. Signal transduction of D1R-like and D2R-like receptors.....	62
Figure 8. The kallikrein-kinin system	70
Figure 9. Sites of proteolytic enzymes of kinins	74
Figure 10. Signaling mechanism of kinin B1R and B2R	95
Figure 11. Diagram of the cardiovascular effects of BK on B2R in the spinal cord	99

Chapter III

Article #1

Figure 1. Identification of the VTA as microinjection site following *post-mortem* histological examination of microinjected Evans'blue. A rat was considered to be correctly injected when a black spot was seen in the VTA without any evidence of haemorrhage or necrosis. Diagram was modified from the atlas of Paxinos and Watson (1998). Abbreviations: VTA, ventral tegmental area; SNR, substantia nigra reticular; ml, medial lemniscus; RMC, red nucleus magnocellular; MS, microinjection site. Scale: 0.5 mm.....

..... 147

Figure 2. Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. (a) or VTA (b) injection of 500 pmol of SB222200 or its inactive enantiomer SB222201. Areas under the curves (AUC) were measured for a period of 0-10 h for i.c.v. and 0-8 h for VTA (small insets). Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF is indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ 148

Figure 3. Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. injection of (a) SB222200 (500 pmol) and (b) R-820 (500 pmol) before and 30 min after treatment with the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v.) or the DA -D₁R antagonist SCH23390 (0.2 mgkg⁻¹, i.v.). Areas under the curves (AUC) were measured for a period of 0-8 h (SB222200) or 0-72 h (R-820) as shown in small insets. Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF values (*) or NK₃R antagonist alone (†) is indicated by * $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$ 149

Figure 4. Time-course effects on changes in mean arterial blood pressure (Δ MAP) (a) and heart rate (Δ H) (b) induced by R-820 (500 pmol) injected into the VTA before and 30 min after treatment with the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v.). Areas under the curves (AUC) were measured for a period of 0-5 h (small insets). Values represent the mean \pm

s.e.m. of n rats. Statistical comparison to aCSF values (*) or R-820 (†) is indicated by * †P<0.05; ** ††P<0.01; *** †††P<0.001. 150

Figure 5. Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. injection of 500 pmol R-820, once daily for a period of 5 days in SHR. Values represent the mean \pm s.e.m. of 4 rats. Statistical comparison to pre-injection values is indicated by *P<0.05; **P<0.01. 151

Figure 6. Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) following i.c.v. (a) or VTA (b) injection of four increasing doses (10, 25, 65 and 100 pmol) of senktide in SHR. Areas under the curves (AUC) were measured for a period of 0-1 h (open bars for VTA and closed bars for i.c.v.). Values represent the mean \pm s.e.m. of 6 rats per injection site. Statistical comparison to aCSF values (*) or i.c.v. (†) is indicated by ** ††P<0.01; *** †††P<0.001. 152

Figure 7. Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) produced by senktide (25 pmol) injected into the VTA prior to (first day) and 1 h after VTA injection of R-820 (second day). Each point represents the means \pm s.e.m. of 4 rats. Statistical comparison to aCSF (*) or senktide (†) values is indicated by * †P<0.05; ** ††P<0.01; *** †††P<0.001 153

Figure 8. Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by i.c.v. senktide 65 pmol (baseline: 161 \pm 5 mmHg) prior to and after treatment with (a) the DA-D₁R antagonist SCH23390 (0.2 mgkg⁻¹ i.v.) and the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹ i.v.) (baseline: 167 \pm 4 mmHg); and (b) the non-selective DA-D₂R antagonist haloperidol (10 mgkg⁻¹ s.c.) (baseline: 164 \pm 2.4 mmHg) in SHR. In (c) are shown the Areas under the curves (AUC) measured for a period of 0-1 h. Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF

(*) or senktide (†) values is indicated by * † $P < 0.05$; ** † $P < 0.01$; *** †† $P < 0.001$ 154

Figure 9. Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by treatments with the DA-D₁R antagonist SCH23390 (0.2 mgkg⁻¹ i.v.), DA-D₂R antagonist raclopride (0.16 mgkg⁻¹ i.v.) and the non-selective DA-D₂R antagonist haloperidol (10 mgkg⁻¹ s.c.) in SHR. Different time scales were used: in (a) (min), and (b) (hours). Values represent the mean \pm s.e.m. of 4 rats. Statistical comparison to saline is indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ 155

Figure 10. Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by senktide injected i.c.v. on the (a) ipsilateral and (b) contralateral side before and after SB222200 (500 pmol) injected into the VTA. The anti-hypertensive effect elicited by the VTA injection of SB222200 (500 pmol) was measured before and after i.c.v. injection of senktide (65 pmol) on the ipsilateral (c) and contralateral (d) side in SHR. Areas under the curves (AUC) for MAP are shown in (e and f). Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF (*) and senktide alone or SB222200 alone (†) is indicated by * † $P < 0.05$; ** † $P < 0.01$; *** †† $P < 0.001$ 156

Figure 11. Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by i.c.v. injected 65 pmol senktide (a, b) or 500 pmol R-820 (c, d) in SHR which underwent an ipsilateral (a, c) and contralateral (b, d) lesion of the VTA with ibotenic acid (IBO), 5 days earlier or a sham-operation. Areas under the curves (AUC) for MAP are shown in (e, f). Each point represents the mean \pm s.e.m of n rats. Statistical comparison to aCSF (*) or sham-operated rats (†) is indicated by * † $P < 0.05$; ** † $P < 0.01$; *** †† $P < 0.001$ 157

Figure 1. Time-course effects on changes in mean arterial blood pressure (Δ MAP) induced by three doses of SSR240612 (1, 5 and 10 mg/kg, gavage) in AngII-treated rats (a). Raclopride (0.16 mg/kg, i.v.) was injected alone or 30 min prior to 5 mg/kg SSR240612 to assess the participation of DA-D2R in the anti-hypertensive effect of SSR240612 (b). Control WKY also received 5 mg/kg SSR240612 (a) or 0.16 mg/kg raclopride (b). Area under the curves (AUC) was measured for a period of 0-6 h (c) or 0-8h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by ***P*<0.01; ***, †††*P*<0.001 181

Figure 2. Time-course effects on changes in mean arterial blood pressure (Δ MAP) induced by three doses of SSR240612 (1, 5 and 10 mg/kg, gavage) in SHR (a). Raclopride (0.16 mg/kg, i.v.) was injected alone or 30 min prior to 5 mg/kg SSR240612 to assess the participation of DA-D2R in the anti-hypertensive effect of SSR240612 (b). Control WKY also received 5 mg/kg SSR240612 (see Fig. 1a) or 0.16 mg/kg raclopride (b). Area under the curves (AUC) was measured for a period of 0-6 h (c,d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by **P*<0.05; ***P*<0.01; ***, †††*P*<0.001..... 182

Figure 3. Time-course effects on changes in mean arterial blood pressure (Δ MAP) induced by SSR240612 (5mg/kg, gavage) in AngII-treated rats (a) and SHR (b) before and 30 min after administration of the dopamine antagonist haloperidol (10 mg/kg, s.c). Hypertensive rats and control WKY also received 10 mg/kg haloperidol alone. Area under the curves (AUC) was measured for a period of 0-8 h (c) or 0-6 h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by **P*<0.05; **, ††*P*<0.01; ***, †††*P*<0.001 183

Figure 4. Time-course effects on changes in mean arterial blood pressure (Δ MAP) induced by SSR240612 (1 μ g, i.c.v.) in AngII-treated rats (a) and SHR (b) before and 30 min after administration of the DA-D2R antagonist raclopride (0.16 mg/kg, i.v.). Control WKY also received i.c.v. SSR240612. Raclopride alone was assessed in previous figures. Area under the curves (AUC) was measured for a period of 0-6 h (c, d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with aCSF values (*) or SSR240612 (†) is indicated by *, †P<0.05; **, ††P<0.01; ***, †††P<0.001..... 184

Figure 5. Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by R-715 and R-954 (2mg/kg, s.c.) in AngII-treated rats (a) and SHR (b). Effects of both B1R antagonists were also tested in control WKY (a). Area under the curves (AUC) was measured for a period of 0-10 h (c) or 0-12 h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) is indicated by **P<0.01; ***P<0.001. 185

Article #3

Table 1. PCR primer pairs used in this study 201

Figure 1. B1R gene expression in hypothalamus, ventral tegmental area (VTA) and nucleus accumbens of SHR and Ang II-treated rats. B1R mRNA levels were measured relative to 18S level. Data represent the mean \pm s.e. mean of values obtained in groups of 4 rats. Statistical comparison to control WKY is indicated by *P<0.05; **P<0.01 or ***P<0.01..... 212

Figure 2. Behavioural responses to 1 μ g Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR and WKY. Each bar represents the mean \pm s.e.mean of values obtained in 4 rats per group. Comparison to WKY is indicated by ***P < 0.001. 213

Figure 3. Inhibition of the behavioural responses to 1 μ g Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR (a, c) and Ang II-treated rats (b, d) before and 3h after treatment with the kinin B1R antagonist SSR240612 administered either i.c.v. (10 μ g) or by gavage (10 mg.kg⁻¹). Each bar represents the mean \pm s.e. mean of values obtained in 4 groups of 6 rats. Comparison to aCSF (*) and to the B1R agonist (†) is indicated by *P<0.05; **†P<0.01; ***P< 0.001..... 214

Figure 4. Inhibition of the behavioural responses to 1 μ g Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR before and 30 min after treatment with the tachykinin NK-1R antagonist RP67580 (a), the NMDA receptor antagonist DL-AP5 (b), the NOS inhibitor L-NNA (c), the iNOS inhibitor 1400W (d), the dopamine D1R antagonist SCH23390 (e), the dopamine D2R antagonist Raclopride (f). Each bar represents the mean \pm s.e. mean of values obtained in 6 groups of 6 rats. Comparison to the B1R agonist before treatment is indicated by *P<0.05, **P<0.01..... 215

Figure 5. Inhibition of the behavioural responses to 1 μ g Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in Ang II-treated rats before and 30 min after treatment with the tachykinin NK-1R antagonist RP67580 (a), the NMDA receptor antagonist DL-AP5 (b), the NOS inhibitor L-NNA (c), the iNOS inhibitor 1400W (d), the dopamine D1R antagonist SCH23390 (e), the dopamine D2R antagonist Raclopride (f). Each bar represents the mean \pm s.e.mean of values obtained in 6 groups of 6 rats. Comparison to the B1R agonist before treatment is indicated by *P<0.05, **P<0.01 216

Figures – General discussion

Figure 12. Schematic representation showing tachykinin NK-3R agonist/antagonists injected i.c.v. (A) and VTA (B) in rats..... 219

Figure 13. Schematic representation of the NK-3R agonist and antagonists in the CNS. In A, upper panel shows that NKB is released and caused high occupancy of NK-3R. DA neurons are excited by NKB, resulting in an increase of DA release in the nucleus accumbens and a high level of DA-D2R occupancy post-synaptically. In B, lower panel illustrates the inhibition of NKB effect by NK-3R antagonist. As consequence, the DA neurons are less excited and the release of DA in the nucleus accumbens is strikingly decreased..... 222

Figure 14. Schematic representation showing the CSF circulation of kinin B1R agonist and antagonists injected i.c.v. in rats 225

Figure 15. Kinin B1R antagonists (SSR240612, R-924, R-715) injected by gavage, i.c.v. and s.c. decreased body temperature in SHR and Ang II-treated rats 236

List of Tables

Table I. Chemical formulae of endogenous tachykinins in mammals.....	10
Table II. Tachykinin NK-1R-selective agonists.....	24
Table III. Chemical formulae of tachykinin NK-1R antagonists.....	25
Table IV. Chemical formulae of tachykinin NK-2R antagonists.....	28
Table V. Tachykinin NK-3R in rat brain.....	32
Table VI. Chemical formulae of tachykinin NK-3R antagonists.....	33
Table VII. Chemical formulae of B1R antagonists	84
Table VIII. Chemical formulae of B2R antagonists.....	90

List of Abbreviations

ACE: angiotensin converting enzyme

ACEI: angiotensin converting enzyme inhibitor

ACE-1: angiotensin converting enzyme-1

Ach: acetylcholine

ADHD: attention deficit hyperactivity disorder

Ang: angiotensin

ANS: autonomic nervous system

ANP: atrial natriuretic peptide

BBB: blood-brain barrier

BK: bradykinin

cAMP: cyclic adenosine monophosphate

cGPM: cyclic guanosine monophosphate

CPM: carboxypeptidase M

CPN: carboxypeptidase N

BP: blood pressure

CGRP: calcitonin gene related peptide

CNS: central nervous system

CREB: cAMP response element binding protein

DA: dopamine or dopaminergic

DA-D1R: dopamine receptor type D1

DA-D2R: dopamine receptor type D2

DAG: diacylglycerol

DOPAC: dihydroxyphenylacetic acid

EGF: epidermal growth factor

GABA: γ -aminobutyric acid

GDNF: glial cell-derived neurotrophic factor

GPCR: G protein-coupled receptors

HR: heart rate

HMWK: high molecular weight kininogen

HPLC: high-performance liquid chromatography

HBECs: human brain endothelial cells

i.c.v.: intracerebroventricular

IP₃: inositol 1,4,5-triphosphate

IRAK: interleukin-1 receptor-associated kinase 1

i.t.: intrathecal

ITIM: immunoreceptor tyrosine-based inhibitory pattern

i.v.: intravenous

KD: kallidin

KO: knockout

LMWK: low molecular weight kininogen

LPS: lipopolysaccharides

MAP: mean arterial blood pressure

MAPK: mitogen-activated protein kinases

mPFC: medial prefrontal cortex

mRNA: messenger ribonucleic acid

NE: noradrenaline

NK- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells

NK-1R: tachykinin receptor type-1
NK-2R: tachykinin receptor type-2
NK-3R: tachykinin receptor type-3
NKA: neurokinin A
NKB: neurokinin B
NO: nitric oxide
NOs: nitric oxide synthases
NPγ: neuropeptide gamma
NPK: neuropeptide K
NTS: nucleus of the solitary tract
PB: parabrachial nucleus
Pa5: paratrigeminal nucleus
PDGF: platelet-derived growth factor
PDTC: pyrrolidine dithiocarbamate
PKA: protein kinase A
PKC: protein kinase C
PLC: phospholipase C
PLD: phospholipase D
PPT: preprotachykinin gene
RT-PCR: polymerase chain reaction
R: receptor
RVLM: rostral ventrolateral medulla
s.c.: subcutaneous
SHR: spontaneously hypertensive rats

SNS: sympathetic nervous system

SN: substantia nigra

SO: supraoptic nucleus of the hypothalamus

SP: substance P

SP5: trigeminal nucleus

STZ: streptozotocin

TRKA: high affinity nerve growth factor

WKY: Wistar-Kyoto rat

VTA: ventral tegmental area

6-OHDA: 6-hydroxydopamine

Chapter I

Introduction and Literature review

1. General introduction

1.0.1 The autonomic nervous system: A strategic role in blood pressure regulation

The autonomic nervous system (ANS) is controlled by central autonomic nuclei of the diencephalon (hypothalamus nuclei), forebrain (amygdala), midbrain (periaqueductal gray region) and brainstem (nucleus of the solitary tract, ambiguous nucleus, and dorsal motor of the vagus, rostral and dorsal ventrolateral medulla). These regions of the central nervous system (CNS) control pre-ganglionic sympathetic and parasympathetic efferent visceromotor fibers (Benarroch, 1993). This system is very complex and provides precise control of cardiovascular function in normal physiological situations (standing, intense exercise).

In addition, the ANS provides a fast and sensitive regulation of arterial pressure due to the baroreflex. Through arterial baroreceptors and chemoreceptors, the cardiovascular centers situated in the brainstem and hypothalamus received constant information on the status of the peripheral circulation (de Champlain, 2001). Several studies supported the major role of ANS in controlling cardiac function and peripheral resistance (de Champlain *et al.*, 1998). Clinical and experimental studies demonstrated that sympathetic activity was increased in hypertensive patients and in several models of experimental hypertension (Julius *et al.*, 1988; de Champlain *et al.*, 1998; Laflamme *et al.*, 1998a, b; Grassi, 1998). Moreover, decreased parasympathetic tone was observed in several cases of clinical and experimental hypertension (Korner, 1989; Korner *et al.*, 1989).

1.0.2 Hypertension

According to 2008 statistics, provided by the Heart and Stroke Foundation of Canada, hypertension affects one in five Canadians. Hypertension is a risk factor that can triple the chance of developing cardiovascular diseases. It is the number one risk factor for stroke and a major risk factor for heart disease. However, since there are no symptoms, 42% of Canadians with high blood pressure (BP) do not even know that they have it. This is why hypertension is known as the silent killer.

The five major classes of anti-hypertensive agents are: diuretics, beta-blockers, calcium channel blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin AT1 receptor antagonists (Plante, 1999).

The causes of hypertension are highly diverse and provide an immense challenge for fundamental research, because it involves the kidneys, vascular, cardiac, nervous and endocrine systems.

1.0.3 The tachykinins and their receptors: regulation of central cardiovascular function in hypertension

Tachykinins are one of the largest families of neuropeptides. They can be found in species from amphibians to mammals. The tachykinin family is characterized by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH₂. Therefore, the tachykinin N-terminal determines the receptor selectivity, while the conserved C-terminal sequence is responsible for the receptor

activation (Lucas *et al.*, 1992). This family is named tachykinins, which means "fast movement" on the contraction of smooth muscles.

In mammals, the family members are substance P (SP), neurokinin A (NKA), neurokinin B (NKB), neuropeptide K (NPK) and neuropeptide γ (NP γ). They are distributed in both the central and peripheral nervous systems. These have a wide range of biological effects that follow the activation of three types of trans-membrane receptors (R) called NK-1, NK-2 and NK-3, which are G protein-coupled receptors (GPCRs). Due to their vast distribution in the CNS, they play a major role in many essential functions such as learning, memory and emotional processes. This also includes stereotyped behaviours, anxiety, stress and pain. Thus, the tachykinins are known to exert central control over many functions of the ANS (Severini *et al.*, 2002).

The potential role of tachykinins in central cardiovascular regulation has been suggested by several studies. Increases in blood pressure and heart rate (HR) accompanied by stereotyped behaviours were shown by tachykinin agonists injected into the i.c.v. and ventral tegmental area (VTA) (Picard *et al.*, 1994; Takano *et al.*, 1990; Cellier *et al.*, 1997; Deschamps and Couture 2005). These cardiovascular effects are dependent on the activation of the sympathetic nervous system as well as the increased release of vasopressin from the neurohypophysis (Unger *et al.*, 1981, Polidori *et al.*, 1989, Takano *et al.*, 1990). However, the specific neuronal circuits involved in cardiovascular and behavioural effects of tachykinins are still largely unknown.

A strategic central dopaminergic (DA) site is the VTA. It is located at the midbrain and composed mainly of dopaminergic neurons forming the area A10. Considering its projections to the limbic system and cerebral cortex, the VTA is more known for its role in the regulation of behavioural activity in response to stress, psychologic diseases, drug addiction and drug withdrawal. However, compelling experimental results suggest its involvement in cardiovascular control. Some studies suggest that the DA system also participates in these events. Indeed, when the three tachykinin NK-1R, NK-2R and NK-3R agonists were injected into the VTA of normotensive rats, they affected the autonomic control of BP and HR by increasing midbrain DA transmission (Deschamps and Couture 2005). Substance P (SP) injected into the i.c.v. improved the baroreflex sensitivity, while the injection of antibodies against SP (into the i.c.v. or directly into the nucleus of the solitary tract (NTS)) reduced the baroreflex (Chan *et al.*, 1990; Appenrodt *et al.*, 1993). Moreover, when SP or a selective NK-1R agonist was injected into the NTS, it induced hypotension and bradycardia. Electrical or chemical stimulation of the VTA with the injection of SP analogue DiMe-C7 produced an increase in BP and locomotor activity. The increases in locomotor activity induced by intra-VTA DiMe-C7 were blocked by the DA-D1R and DA-D2R antagonists, SCH23390 and haloperidol, respectively (Kubos *et al.*, 1987; Cornish *et al.*, 1994; Placenza *et al.*, 2004). When the NK-3R agonist senktide was injected into the VTA, it caused several behaviours such as yawning and chewing known to be evoked by DA (Stoessl *et al.*, 1991). Furthermore, the

activation of NK-3R by endogenous tachykinins in the substantia nigra (SN), an important DA center, stimulated the cardiac function. The NK-3R antagonist (SB222200) significantly reduced mean arterial pressure (MAP) for more than three hours when injected into the SN in spontaneously hypertensive rats (SHR) (Lessard *et al.*, 2001, 2003, 2004). However, the function of NK-3R in the CNS during pathophysiological situations remains poorly understood.

1.0.4 The kinins and their receptors: regulation of central cardiovascular function in hypertension

Kinins are a small family of peptides (9 to 11 amino acids), including bradykinin (BK), kallidin (KD; Lys-BK) and T-kinin (Ile-Ser-BK) (Gabra *et al.*, 2003). The most studied members of this family are BK and KD. Their active metabolites are produced by the enzymatic action of kininase I (des-Arg⁹-BK, des-Arg¹⁰-KD). These peptides produced their biological effects via the activation of two types of GPCRs, named B1 and B2. The B2R is activated by BK, KD and T-kinin (rats only) while the B1R is activated by des-Arg⁹-BK and des-Arg¹⁰-KD (Leeb-Lundberg, 2001). As opposed to B2R, which is expressed in most tissues, the B1R is usually absent in healthy tissues. The B1R is inducible and its expression is increased in the presence of cytokines, bacterial lipopolysaccharides (LPS) and after tissue injury (Marceau *et al.*, 1998b).

Kinins have been identified in the CNS and sufficient evidence suggests that kinins play a role as neuromodulators in central cardiovascular regulation, pain and inflammation (Couture and Lindsey, 2000; Couture *et al.*, 2001). In freely behaving normotensive rats, BK caused a pressor response when it was injected into the i.c.v. or directly into the NTS and paratrigeminal nucleus (Pa5) (Corrêa and Graeff, 1974; Fior *et al.*, 1993; Lindsey *et al.*, 1997; Couture and Lindsey 2000).

The thoracic spinal cord is another site of central cardiovascular regulation. Previous studies from our laboratory have shown that BK injected intrathecally (i.t.) increased BP via the activation of the sympatho-adrenal system and B2R (Lopes *et al.*, 1992; Lopes *et al.*, 1993). This was substantiated by autoradiographic studies, which revealed the presence of B2R binding sites in the spinal cord (Lopes *et al.*, 1995; Couture and Lindsey, 2000).

Emanuelli *et al.* (1999) reported that B1R agonists injected into the i.c.v. in SHR and Wistar Kyoto rats (WKY) caused increases in BP, while the B1R antagonist R-715 caused a small decrease of BP in SHR. The latter findings were not reproduced with the identical B1R agonists and antagonists in SHR (Cloutier *et al.*, 2004). Thus, the role of central kinin B1R in hypertensive rats is still controversial.

1.1 Tachykinins

The name tachykinin resulted from the pharmacological similarity of these peptides to bradykinin. The tachykinins, while having generally the same

pharmacological profile, induced a fast contraction of the tissue (Khawaja and Rogres, 1996).

Tachykinins are present in several peripheral tissues where they perform many functions on the cardiovascular, respiratory and gastro-intestinal systems. Their effects include vasodilatation, increased vascular permeability, bronchoconstriction and contraction of smooth muscle cells. Neurogenic inflammation involved in diseases such as asthma is induced following the release of tachykinins from peripheral sensory C-fibers (Harrison and Geppetti, 2001).

The discovery of SP, by von Euler and Gaddum in 1931, introduced a new era of peptides in neuroscience. This previously unidentified substance was found in alcoholic extracts of the equine brain and horse intestine. It was isolated and characterized as an eleven amino acid peptide, which had a potent stimulant action on the jejunum and also caused hypotension (Severini *et al.*, 2002).

Later, neuromedin K and neuromedin L were isolated from the porcine spinal cord (Kangawa *et al.*, 1983; Minamino *et al.*, 1984) and neurokinin α and neurokinin β from guinea-pig ileum and rat duodenum (Kimura *et al.*, 1984). Substance K, neurokinin α , and neuromedin L were re-named neurokinin A (NKA), while neurokinin β and neuromedin K were called neurokinin B (NKB) (Maggi, 2000).

Two other mammalian tachykinins, neuropeptide K and neuropeptide γ , were isolated from porcine brain (Tatemoto *et al.*, 1985) and the rabbit

intestine (Kage *et al.*, 1988), respectively. These last two tachykinins were the products of the same gene (preprotachykinin A), which encodes SP and NKA. They were identified as the N-terminal elongated forms of NKA (Table 1).

Table I. Chemical formulae of endogenous tachykinins in mammals

	Sequences	References
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂	Chang and Leeman, 1970
NKA	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂	Nawa <i>et al.</i> , 1984a
NKB	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH ₂	Kangawa <i>et al.</i> , 1983
Neuropeptide K	Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Lys- Ala-Leu-Tyr-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys- Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂	Tatemoto <i>et al.</i> , 1985
Neuropeptide γ	Asp-Ala-Gly-Tyr-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr- Asp-Ser- Phe-Val-Gly-Leu-Met-NH ₂	Kage <i>et al.</i> , 1988
Hemokinin-1	Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Met-NH ₂	Zhang <i>et al.</i> , 2000
Endokinin	Gly-Lys-Ala-Ser-Gln-Phe-Phe-Gly-Leu-Met-NH ₂	Page <i>et al.</i> , 2003

1.1.2 Tachykinins: biosynthesis and distribution

Tachykinins were encoded by two genes that, according to the Human Genome Organization Gene Nomenclature Committee, are termed TAC1 (SP, NKA) and TAC3 (NKB), which replace the previously used terms PPT-A and PPT-B (Patacchini *et al.*, 2004).

The precursor RNA from TAC1 was alternatively processed to yield four different mRNAs (α , β , γ and δ) (Figure 1). SP was encoded by all four mRNAs. NKA was encoded by β - and γ -PPT, neuropeptide K by β -PPT, and neuropeptide- γ by γ -PPT. The TAC3 gene encoded the precursor for NKB, which derived from α - and β -mRNAs (Severini *et al.*, 2002). Recently, TAC4, a third tachykinin gene preprotachykinin-c (PPT-C) has been discovered in lymphoid B hematopoietic cells of mouse bone marrow (Zhang *et al.*, 2000). TAC4 encoded hemokinin-1 and endokinin (Page, 2004). The expression of TAC1 and TAC3 genes which vary between tissues and species determined the distribution of the classical tachykinins (Page, 2005). Although the β -TAC1 mRNA was the predominant type expressed in the human basal ganglia, α -TAC1 mRNA was abundant in the mammalian brain (Bannon *et al.*, 1992). The β - and γ - TAC1 mRNAs were found mainly in peripheral tissues (Nakanishi, 1987).

The most studied member among the tachykinins is unquestionably SP. The distribution of SP is greatly expressed in CNS areas that are involved in the regulation of pain transmission, affective behaviour and stress (De Felipe *et al.*, 1998; Kramer *et al.*, 1998).

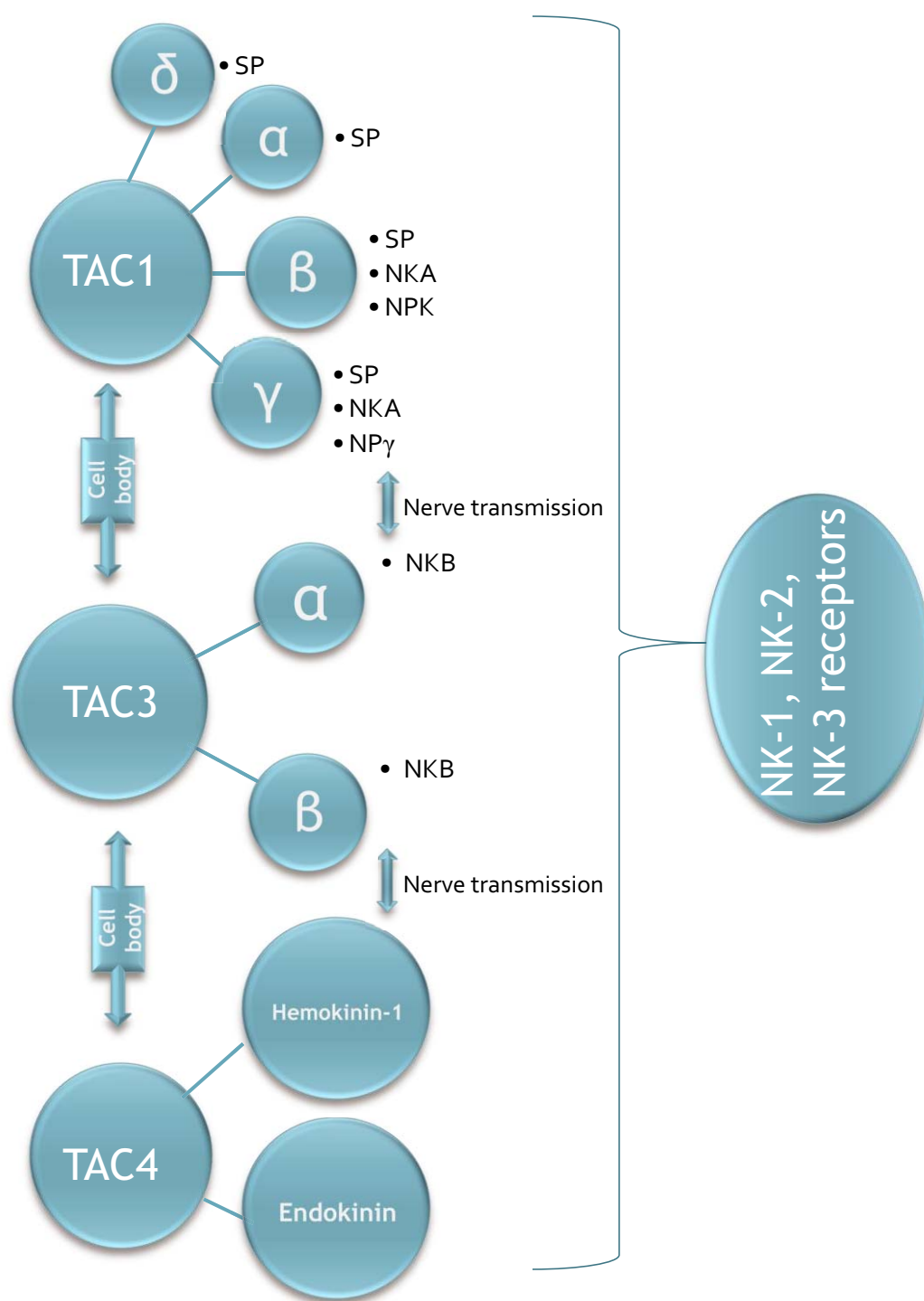


Figure 1. **Schematic representation of the TAC1, TAC3, and TAC4 genes expression and their mRNA messengers.** *Adapted from Guard and Watson, 1991.*

SP is present in the limbic system, including the hypothalamus and the amygdala, areas associated with emotional behaviour (Stahl, 1999). In the mesolimbic brain areas, SP is believed to be involved in reward, opioids withdrawal (Murtra *et al.*, 2000) and memory-promoting effects (Huston and Hasenohrl, 1995). Immunoreactive-SP nerve fibers were found in the rat cortex and hippocampus, and were lightly distributed all over the layers of the neocortex. Studies revealed that the localization of these cells was a subset of the glutamic acid decarboxylase containing neurons supposedly γ -aminobutyric (GABA)-ergic neurons (Penny *et al.*, 1986). In some neurons of the raphe nuclei of the rat medulla, SP co-existed with serotonin and GABA (Chan-Palay, 1978; Magoul *et al.*, 1986).

SP has been found in dorsal root ganglia and sensory neurons as well as in the most autonomic ganglia and intrinsic enteric neurons in the peripheral nervous system (Hokfelt *et al.*, 1977). The highest concentrations of immunoreactive-SP fibers were found in the SN and dorsal horn of the spinal cord and in nerve terminals of the hypothalamus in rats (Figure 2) (Kanazawa and Jessell, 1976; Douglas *et al.*, 1982; Ljungdahl *et al.*, 1978). Substantial, but lower levels were present in other regions including amygdala, caudate putamen, and globus pallidus (Kertes *et al.*, 2009). The distribution of immunoreactive-SP neurons and fibers in the human brain showed considerable similarity to the rat brain, including dense distribution of immunoreactive-SP in the SN, the caudate putamen, and the

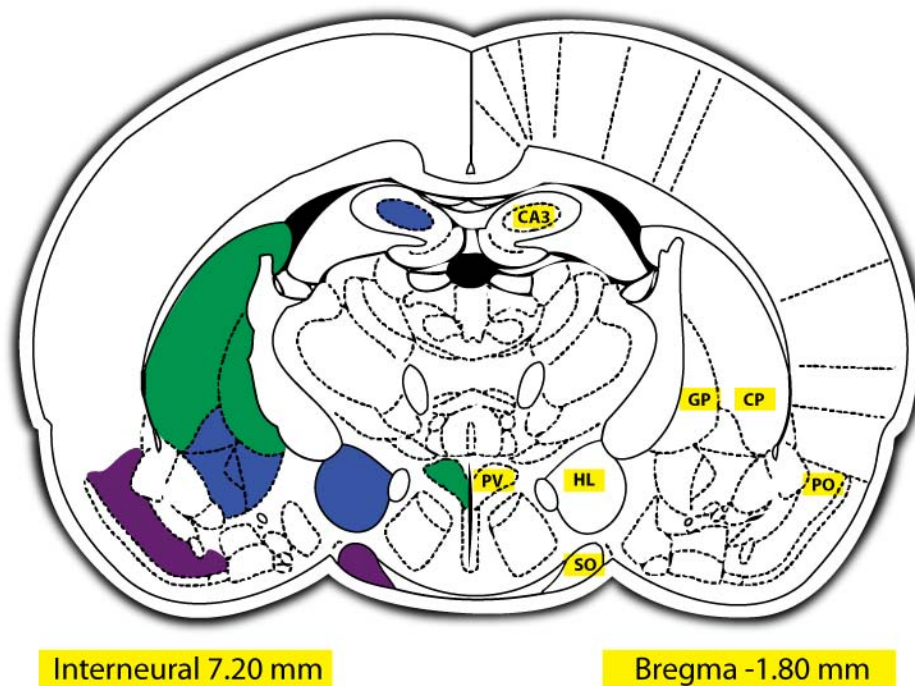


Figure 2: Distribution of immunoreactive-SP in the rat hypothalamus (bregma: -1.8 mm (Paxinos and Watson, 1998). Scale of density: green, high; blue, moderate and plum, low. CA3, CA3 region of the hippocampus; CP, caudate nucleus and putamen of the striatum, GP, globus pallidus; HL, lateral hypothalamus; PV, paraventricular nucleus of the hypothalamus; PO, piriform cortex; SO, supraoptic nucleus of the hypothalamus. Adapted from Shults *et al.*, 1984.

brain stem (Chahl, 2006). However, the human cortex and hippocampus contained more abundant SP cells and fibers than rat brain (Hokfelt *et al.*, 1976; Mai *et al.*, 1986; Del Fiacco *et al.*, 1987). The other classical tachykinins were generally found in lower concentrations than SP in the CNS. Most studies on the distribution of tachykinins have used antisera directed towards the C-terminal region of the peptides which differentiate weakly between the family members. The recent discovery of hemokinin-1 and endokinin and their cross-reactivity with anti-SP antibodies were questioning several of the older studies on the peripheral and central distribution of the classical tachykinins (Page, 2004). Recent studies using molecular biological techniques have confirmed that TAC1 and TAC3 mRNAs were strongly expressed in the brain (Pinto *et al.*, 2004) and peripheral tissues, as described in previous studies. Since the classical tachykinins were synthesized in the cell soma and transported to the nerve terminals (Krause *et al.*, 1987), precursor mRNAs were present in the cell soma, whereas the peptides were predominantly present in the nerve terminals. Thus, immunocytochemical methods will detect tachykinins in tissues that do not necessarily contain the mRNAs.

SP has been shown to preferentially activate mesocortical DA neurons in a similar way (Tamiya *et al.*, 1990). SP injected directly into the VTA increased the levels of DA, its metabolites or both, in the prefrontal cortex and the nucleus accumbens, suggesting that SP increased the release of DA from mesocortical and mesolimbic DA neurons (Deutch *et al.*, 1985; Cador *et al.*, 1989; Barnes *et al.*, 1990; Elliott *et al.*, 1992).

SP has been shown to preferentially activate mesocortical DA neurons in a similar manner to acute stressors such as mild foot shock or restraint (Elliott *et al.*, 1986b). Furthermore, evidence suggested that the increased turnover of DA in the prefrontal cortex and the nucleus accumbens in response to stress may have been mediated by SP. Increases in DA metabolism in the prefrontal cortex in response to foot shock stress was blocked by an SP antibody in the VTA (Bannon *et al.*, 1983). These findings suggested that SP not only activated mesocorticolimbic DA neurons, but the activation of these DA neurons in response to stress may have been mediated by the endogenous SP system. The activation of VTA DA neurons has been implicated in the mediation of relapse, mainly in drug-induced (McFarland and Kalivas, 2001), and perhaps foot shock-induced relapse as well (Capriles *et al.*, 2003).

1.2 The NKA and NKB

Immunohistochemical studies and *in situ* hybridization showed a high distribution of NKA, NKB and their mRNA in each major subdivision of the brain (Minamino *et al.*, 1984; Nagashima *et al.*, 1989; Nawa *et al.*, 1984b), including the hypothalamus (Tateishi *et al.*, 1989; Larsen, 1992; Merchenthaler *et al.*, 1992), midbrain (Arai and Emson, 1986) cerebral cortex and medulla (Minamino *et al.*, 1984). NKA and SP were both localized to the same region. This was consistent with the fact that these two neuropeptides were synthesized from the same precursor (Ribeiro-da-Silva *et al.*, 2000).

In humans and monkeys, the mRNA gene expression of the TAC1 responsible for the synthesis of SP and NKA was detected in the striatum, hypothalamus, periaqueductal gray region and the amygdala (Arai and Emson, 1986; Larsen *et al.*, 1992; Sergeyev *et al.*, 1999).

Immunocytochemical studies revealed high density of NKB, in contrast to a low density of SP, in the olfactory bulb, cerebral cortex and hippocampus in rats (Lucas *et al.*, 1992). Although the raphe nucleus possesses a high density of SP, NKB was not detected (Warden and Young, 1988). In addition to these regions, NKB was found in the striatum and several nuclei of the hypothalamus, including the lateral and paraventricular nucleus (Merchenthaler *et al.*, 1992).

The gene expression of c-fos was upregulated in rat brain areas activated by tachykinins (Spitznagel *et al.*, 2001). Increased numbers of c-fos positive neurons were detected in the septum, amygdala, paraventricular nucleus, supraoptic nucleus, lateral hypothalamus, thalamus, VTA, and SN following i.c.v. injection in rats of NKB or senktide, a NK-3R agonist (Smith and Flynn, 2000). Using the same approach in rat, i.c.v. injected SP showed small expression of c-fos in the paraventricular and mediodorsal nuclei of the hypothalamus, the parabrachial nucleus and the medial thalamus. C-fos expression was reduced significantly in the presence of tachykinin NK-1R antagonist, RP67580 (Spitznagel *et al.*, 2001). It is tempting to suggest that SP and NKB may activate distinct neural pathways, and consequently, that they are involved in different physiological functions. Further studies are necessary to confirm this hypothesis.

1.3 Tachykinin receptors

The existence of multiple receptors for tachykinins emerged with the work of Erspamer *et al.* (1981) showing considerable differences between the pharmacological profiles of tachykinins. The distribution of tachykinin receptors has been examined after identification and molecular cloning of the three tachykinin receptors (Yokota *et al.*, 1989; Hershey and Krause, 1990; Nakanishi, 1991).

The three tachykinin receptors (NK-1, NK-2 and NK-3) have a very high homology between them (from 53.7 to 66.3%) in the seven transmembrane domains cytoplasmic or intracellular regions (Shigemoto *et al.*, 1990). Lee *et al.* (1982) were the first to suggest the existence of several types of receptors for tachykinins.

The criteria originally used to distinguish the three receptors were; (1) the order of affinity of mammalian and non-mammalian tachykinins; (2) the activity induced by different fragments of tachykinins and, (3) the development of selective agonists (Maggi, 1995). Thanks to the first generation of SP antagonists and subsequently, to the discovery of NKA and NKB, the existence of three distinct receptors was suggested and later confirmed by molecular cloning. These receptors belonged to the “class A” family of seven-transmembranes G-protein-coupled receptors. It was not until 1986, at an international symposium held in Montreal on tachykinins, until the official nomenclature became neurokinin-1 (NK-1), neurokinin-2 (NK-2) and neurokinin-3 (NK-3) (Henry *et al.*, 1987).

All endogenous tachykinins can act as full agonists at the three receptors, yet SP and hemokinin-1 are preferential agonists at NK-1 receptors (R), whereas NKA (NKA) and NKB (NKB) are preferential agonists at NK-2R and NK-3R, respectively (Regoli *et al.*, 1994; Patacchini *et al.*, 2004).

1.4 Tachykinins: agonists and antagonists

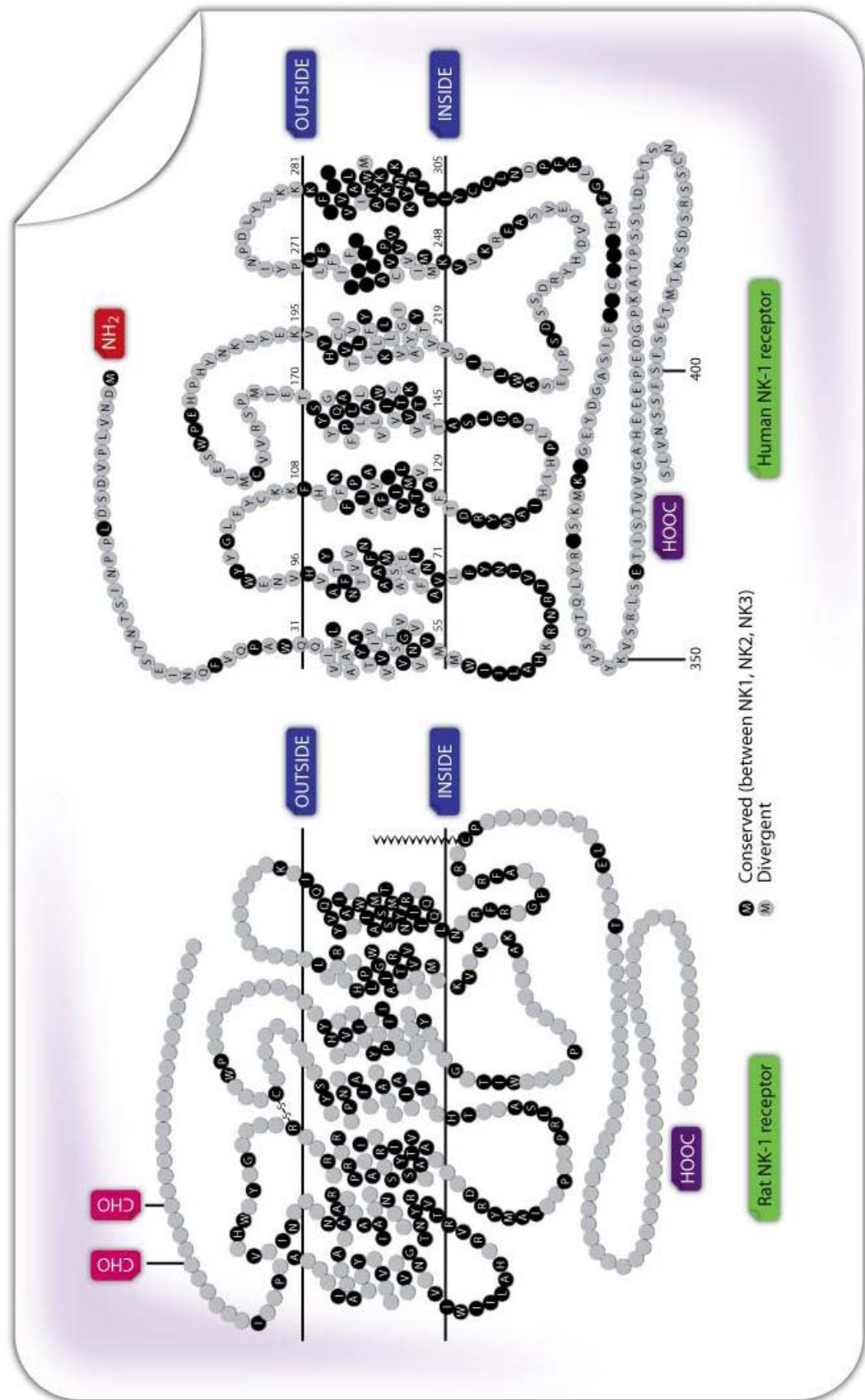
1.4.1 NK-1R

The first tachykinin receptor to be cloned was the NK-1R from the brain and the submandibular gland of the rat (Yokota *et al.*, 1989; Hershey and Krause, 1990). It was identified in neurons and glial cells in the rat CNS (Buck *et al.*, 1986), and in human amygdala and forebrain (Weidenhofer *et al.*, 2006; Draganic *et al.*, 2007) (Figure 3).

NK-1R was highly expressed in the hypothalamus, pituitary gland and amygdala, which are the brain regions that are critical for the regulation of affective behaviour and neurochemical responses to stress (Kramer *et al.*, 1998).

Furthermore, the neural pathways that respond to stress, noxious or aversive stimulation were associated with the amygdala (Kramer *et al.*, 1998).

Figure 3: Schematic representation of the rat and human tachykinin NK-1R.



Adapted from Regoli et al., 1994.

The important role of NK-1R in neurogenic inflammation has been confirmed by studies using NK-1R knockout (KO) mice (Cao and Rodgers, 1998). The observation that tachykinin expression in both sensory neurons and hematopoietic cells is needed for the development of inflammation following antigen-antibody complex formation, at least in the airways, has been highlighted by the availability of the SP/NKA KO-mice (Chavolla-Calderon *et al.*, 2003).

Preclinical research has implicated NK-1R in several pathological disorders, including emesis, asthma, psychiatric and gastrointestinal disorders, pain, migraine, inflammation and urinary bladder disorders (Lindstrom *et al.*, 2007).

The oxidations of methionine at the C-terminal as well as the N-methylation of residue Gly9 of SP have greatly improved the selectivity of the natural peptide to NK-1R (Mussapi *et al.*, 1993). The [Sar⁹, Met (O₂)¹¹]-SP was a relatively stable agonist with a high affinity and selectivity for the NK-1R (Regoli *et al.*, 1988). The existence of a subtype of NK-1R has been suggested in several studies; at least some agonists such as septide can bind to dissimilar sites of the SP receptor. It was proposed that these binding sites were located on different conformers at the NK-1R (Beaujouan *et al.*, 2000). Agonists that have a pharmacological profile similar to SP were therefore, called "classical" while the agonists, which showed a similar profile to septide were called "septide-like agonists" (Maggi, 1995) (Table II). The first selective peptide antagonist for NK-1R was L-668169 developed in the late 1980s. However, like many others that

followed, this antagonist had low affinity and selectivity and displayed side effects (Maggi, 1995).

Other peptide antagonists (FK888 and Cam-2445) have subsequently been developed with higher affinity and selectivity (Fujii *et al.*, 1992; Chan *et al.*, 1996) (Table III). To increase the resistance of the antagonists to tissue peptidases, CP-96345, the first non-peptide antagonist, was developed (Snider *et al.*, 1991). Conversely, it had a very low affinity for the NK-1R in mouse and rat and induced several side effects, among others, on the cardiovascular system and neural transmission by interacting with ionic channels (Wang and Hakanson, 1992).

RP67580, widely used in rats and mice to evaluate the physiological roles of NK-1R, has also some side effects including blocking calcium channels (Rupniak *et al.*, 1993). In order to reduce nonspecific effects while maintaining a high affinity and selectivity, other non-peptide antagonists were developed such as LY303870, a potent antagonist (Iyengar *et al.*, 1997) (Table III).

The possibility that NK-1R antagonists could be valuable as antipsychotic drugs was addressed based on evidence that SP modulates the activity of the mesolimbic DA system, which is the site of action of antipsychotic drugs. Consistent with this interpretation, the locomotor hyperactivity and changes in accumbens cell firing induced by intra-VTA infusion of SP were blocked by the DA-receptor antagonist haloperidol, an antipsychotic drug (Elliott *et al.*, 1991). However, the lack of effect for the NK-1R antagonists in

these studies suggested that the effects of SP in rat VTA was mediated by the stimulation of NK-3R, rather than NK-1R, as suggested by anatomical (Saffroy *et al.*, 1988), electrophysiological (Seabrook *et al.*, 1995) and behavioural studies (Stoessl *et al.*, 1991). Additionally, an exploratory trial with MK-869, which is a non-peptide antagonist with a high affinity and selectivity for the human NK-1R, showed a lack of efficacy in schizophrenic patients (Kramer *et al.*, 1998; Rupniak *et al.*, 1999).

To date, little is known about the way antagonists interact with NK-1R and even less is known about the mechanisms that govern the duration of their effects *in vivo*. The *in vivo* efficacy of an antagonist and its duration of action can sometimes be difficult to predict based only on potency values obtained by *in vitro* assays (Copeland *et al.*, 2006)

Table II. Tachykinin NK-1R-selective agonists

Classical agonists	"Septide-like agonists"
[Pro ⁹]SP	[Glp ⁶ , Pro ⁹]SP (septide)
[Pro ⁹]SP sulfone	SPOMe
[Sar ⁹]SP sulfone	[Apa ⁹⁻¹⁰]SP
Physalaemin	[Pro ⁹⁻¹⁰]SP
[Gly ⁹ Ψ(CH ₂ CH ₂)-Leu ¹⁰]SP	[Glu(OBz) ¹¹]SP
	γ-aminovalery [pro ⁹ , NMeLeu ¹⁰]SP(7-11) (GR73,632)
	[Gly ⁹ Ψ(CH ₂ CH ₂)-Gly ¹⁰]SP
	γ-aminovalery [Pro ⁹ , NMeLeu ¹⁰]SP(7-11)

Adapted from Maggi, 1995.

Table III. Chemical formulae of tachykinin NK-1R antagonists

Antagonists	Sequences	References
L-668169	Cyclo(Gln,DTrp,(NMe)Phe(R)Gly[ANC-2]Leu,Met	Maggi, 1995
FK 888	(2-(N-Me)indolil)-CO-Hyp-Nal-NMeBzl	Maggi, 1995
Cam-2445	[4-Me]Z-(R)uMeTrpNH(S)CHMePh	Maggi, 1995
CP-96345	(2S, 3S)-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-met1-azabicyclo[2.2.2]octan-3-amine	Snider <i>et al.</i> , 1991
RP67580	(3aR,7aR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one	Garret <i>et al.</i> , 1991
LY 303870	(R)-1-[N-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[N-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino] propane	Iyengar <i>et al.</i> , 1997

1.4.2 NK-2R

The NK-2R was cloned from the bovine stomach and rat brain (Masu *et al.*, 1987; Sasai and Nakanishi, 1989). It had 48% homology with the NK-1R (Yokota *et al.*, 1989). The distribution of NK-2R has been studied mainly in the periphery, where high densities of binding sites were found (Mantyh *et al.*, 1989; Tsuchida *et al.*, 1990). These receptors have been localized on astroglial cells in rat spinal cord using electron microscopy (Zerari *et al.*, 1998). However, the NK-2R were also located in myenteric of neurons descending colon of rats (Mantyh *et al.*, 1989). The presence of NK-2R in the CNS has long been disputed; even autoradiography could not unequivocally prove their existence (Bergstrom *et al.*, 1987; Saffroy *et al.*, 1988; Mantyh *et al.*, 1989; Nakanishi, 1991). Conversely, other studies have reported the existence of NK-2R at low concentrations in the striatum, hippocampus, hypothalamus, cerebral cortex and SN of rats and humans, by measuring mRNA by in situ hybridization or RT-PCR (Whitty *et al.*, 1995; Bensaid *et al.*, 2001). Moreover, autoradiography studies using as radioligand [3H]-SR48968 (a selective non-peptide antagonist NK-2R) showed the presence of NK-2R in discrete brain areas, including frontal cortex, hippocampus, amygdala, striatum, hypothalamus, thalamus and SN in rats (Saffroy *et al.*, 2001, 2003). Several NK-2R agonists were developed based on observations that the fragment NKA (4-10) was more active and selective than NKA for the NK-2R (Drapeau *et al.*, 1987). The selectivity of NKA (4-10) was improved by replacing the residue Met¹¹ by Nle, ([Nle¹⁰]-NKA (4-10)) or by replacing the residue Gly⁸ by the β -Ala

residue which yielded the selective agonist [β -Ala⁸]-NKA (4-10) (Rovero *et al.*, 1989). The agonists [Lys⁵, MeLeu⁹, Nle¹⁰]-NKA (4-10) and [Lys³, Gly⁸-R- γ -lactam-Leu⁹]-NKA (3-10) (GR-64349) also have a high affinity and selectivity for NK-2R (Chassaing *et al.*, 1991, Hagan *et al.*, 1991).

The cyclic hexapeptide L-659877 was one of the first antagonists with high affinity and selectivity for the NK-2R. MEN10627 was synthesized in order to optimize the properties of L-659877. Polycyclic hexapeptides were more stable and retained the active conformation. MEN10627 was a competitive and reversible antagonist with potency comparable to SR48968 (Maggi *et al.*, 1994). SR48968 was the first non-peptide antagonist developed to have a very high affinity and selectivity for the NK-2R. SR48968 was also a competitive antagonist also known for its long duration *in vivo* (Advenier *et al.*, 1992; Emonds-Alt *et al.*, 1992). GR-159897 was another good non-peptide NK-2R antagonist (Beresford *et al.*, 1995) (Table IV).

Table IV. Chemical formulae of tachykinin NK-2R antagonists

Antagonists	Sequences	References
L-659877	Cyclo(Gln-Trp-Phe-Gly-Leu-Met)	Maggi <i>et al.</i> , 1992
MEN10627	Cyclo(Met-Asp-Trp-Phe-Dap-Leu)cyclo(2 beta-5 beta)	Maggi <i>et al.</i> , 1994
SR 48968	(S)-N-methyl-N-[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl]benzamide	Advenier <i>et al.</i> , 1992
GR-159897	[(R)- 1-[2-(5-fluoro-1 <i>H</i> -indol-3-yl)ethyl]-4-methoxy-4[(phenylsulfinyl)- methyl]piperidine]	Beresford <i>et al.</i> , 1995

1.4.3 NK-3R

The NK-3R was cloned shortly after the NK-1R and NK-2R (Shigemoto *et al.*, 1990). NKB is the endogenous ligand that has the greatest affinity for the NK-3R. The rank order of affinity of tachykinins for the NK-3R were NKB>>NKA>SP (Smith and Dawson, 2008).

Studies of function and localization have demonstrated the presence of NK-3R in mammalian central, peripheral and enteric nervous systems where its activation influences the release of a variety of neurotransmitters (for review, see Maggi, 1995). NK-3R were expressed in almost the entire CNS and spinal cord in rats (Almeida *et al.*, 2004).

NK-3R was located in the medial prefrontal cortex (mPFC), thalamus, and amygdala. It was present in the midbrain DA nuclei (VTA) and SN, locus coeruleus, and septal and basal nuclei, which suggested its role in modulating central monoaminergic systems (Langlois *et al.*, 2001). NK-3R was also present in dopamine (DA), and non-DA neurons of the VTA (Chen *et al.*, 1998; Lessard *et al.*, 2007).

NK-3R were located in mesolimbic and mesocortical pathways of VTA, which were known to be related to behaviours and cognitive functions, as well as to the pathophysiology of schizophrenia (Spooren *et al.*, 2005; Lessard *et al.*, 2009). This is suggested, in part, by the clinical efficacy of two non-peptide NK-3R antagonists (osanetant and talnetant) on the positive symptoms of schizophrenia (Meltzer, 2004; Spooren *et al.*, 2005). This idea was also consistent with the hyperactivity of mesolimbic DA neurons in schizophrenia and the NK-3R was also distributed in other brain areas

(Table V). Activation of NK-3R located on the cell body of presynaptic neurons may have enhanced DA, acetylcholine (ACh), norepinephrine (NE) and GABA efflux at the nerve terminals (Marco *et al.*, 1998; Jung and Bennett, 1996; Preston *et al.*, 2000). These data suggested that NK-3R may be a target for treatment of psychiatric disorders (for review see Spoooren *et al.*, 2005).

Several peptide and non-peptide NK-3R agonists and antagonists have been developed. Selectivity and affinity for NK-3R were noticeably increased once the agonist [MePhe⁷]-NKB (4-10) replaced Val⁷ with MePhe in the fragment NKB (4-10) (Drapeau *et al.*, 1987). Another agonist that also had a good affinity for the NK-3R were the [Pro⁷]-NKB. However, senktide [(succinyl-[Asp⁹, MePhe⁸])-SP(6-11)] was considered the best NK-3R agonist due to its high affinity and selectivity. Indeed, to activate NK-1R or NK-2R, the concentration of senktide had to be at least 60,000 times stronger than that required to activate the NK-3R (Wormser *et al.*, 1986). R-486 and R-487 belonged to the first generation of antagonists selective for NK-3R, yet they maintained agonist activity to NK-1R and NK-2R (Drapeau *et al.*, 1990) (Table VI). Being more selective, R-820 was a semi-peptide antagonist that had a good affinity in rats (Regoli *et al.*, 1994). The first human non-peptide selective NK-3R antagonist was SR142801 (Emonds-Alt *et al.*, 1995; Oury-Donat *et al.*, 1995). However, in rat SR142801 and its (R)-enantiomer (SR 142806) act as agonists at spinal and supra-spinal levels (Cellier *et al.*, 1997; Couture *et al.*, 2000). SB222200 had a very

high affinity and selectivity for NK-3R in rat and mouse, and it crosses the blood-brain barrier (BBB) (Sarau *et al.*, 2000).

Table V. Tachykinin NK-3R in rat brain

Brain structure	Relative expression
Cerebral cortex	
Cingulate cortex	+++
Frontoparietal	++(+)
Entorhinal cortex	+
Piriform cortex	+
Amygdala	
Basal nucleus	++(+)
Central nucleus	++
Lateral nucleus	+(+)
Hippocampus	
CA1–CA3	+(+)
Dentate gyrus	++
Subiculum	++(+)
Mesencephalon	
Ventral tegmental area	+++
Substantia nigra	+++
pars compacta	
Dorsal raphe nucleus	+(+)
Metencephalon	
Locus coeruleus	+(+)

The relative expression of tachykinin NK-3R in different rat brain areas. Achieving: +, low expression; ++, moderate expression; +++, high expression. *Adapted from Spooren et al., 2005.*

Table VI. Chemical formulae of tachykinin NK-3R antagonists

Antagonists	Sequences	References
R-486	H-Asp-Ser-Phe-Trp- β -Ala-Leu-Met-NH ₂	Drapeau <i>et al.</i> , 1990
R-487	H-Asp-Ser-Phe-Phe-Ala-Leu-Met-NH ₂	Drapeau <i>et al.</i> , 1990
R-820	3-Indolyl-carbonyl-Hyp-Phg-N(Me)-Bzl	Regoli <i>et al.</i> , 1994
SR142801	(S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide	Beaujouan <i>et al.</i> , 1997
SB222200	(S)-(-)-N-(α -ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide]	Sarau <i>et al.</i> , 2000
SB235375	(-)-(S)-N-(α -ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide	Hay <i>et al.</i> , 2002
SB223412	(S)-(-)-N-(α -ethylbenzyl)-3-hydroxy-2-phenylquinoline-4-carboxamide	Smith <i>et al.</i> , 2009
GSK172981	3-amino-N-[(S)-cyclopropyl (phenyl)methyl]-2-(3-fluorophenyl)-4-quinolinecarboxamide	Dawson <i>et al.</i> , 2010
GSK256471	N-[(S)-cyclopropyl (3-fluorophenyl)methyl]-3-[[methyl(methylsulfonyl)amino]methyl]-2-phenyl-4-quinolinecarboxamide	Dawson <i>et al.</i> , 2010

Nevertheless, its low solubility limited its use (Sarau *et al.*, 2000). More recently, SB235375, a water soluble non-peptide antagonist, was developed; it showed good affinity and selectivity, particularly to NK-3R in the guinea pig (Hay *et al.*, 2002). Talnetant (SB223412) was a member of a new class of potent, competitive and selective non-peptide NK-3R antagonists that were based on the 2-phenylquinoline backbone (Table VI).

1.5 Molecular biology of tachykinin receptors

The tachykinin NK-1R, NK-2R and NK-3R have been cloned in several animal species and humans. In rats, the three receptors were respectively constituted of 407, 390 and 452 amino acids (Gerard *et al.*, 1993). However, all the three receptors possess potential N-glycosylation sites at the extracellular amino-terminal and many serine and threonine residues, which are likely phosphorylation sites at the cytoplasmic carboxyl-terminal. Consequently, all the three tachykinin receptors belong to the GPCRs family and have seven transmembrane domains (Nakanishi, 1991). The amino acid sequences in transmembrane domains and adjacent cytoplasmic portions showed similarity of 54-66% between the NK-1R, NK-2R and NK-3R (Shigemoto *et al.*, 1990). In addition, all three receptors contain a histidine residue on the transmembrane segments V and VI, which were characteristic of tachykinin receptors. The third cytoplasmic loop as well as parts for the C-terminal was highly conserved between NK-1R and NK-3R, which was not the case for the NK-2R (Ohkubo and Nakanishi, 1991).

The homology between tachykinin receptors in rats, mice, guinea pigs and humans showed that only 10% of amino acids allowed differentiating the NK-1R between these species. In fact, only 23 amino acids were different between the NK-1R in humans and rats, and only nine amino acids were different between rats and mice.

In rats and mice, unlike humans and guinea pigs, a fourth potential glycosylation site at the position 190 in the second extracellular loop between M4 and M5 has been identified in the sequence of the receptor. This could explain the pharmacological differences observed between species. For the NK-2R, a homology of 85% was observed between the species mentioned above. The NK-3R, NK-1R and NK-2R differed by an extension of 40-50 amino acids in the sequence in rats and humans. However, as the NK-1R and NK-2R, these receptors were highly conserved between rats and humans with a difference of only 12% of amino acids (for review, see Gerard *et al.*, 1993).

1.6 Intracellular signaling pathways

The G-protein includes three different sub-units, named α , β and γ , which are bound to a guanosine diphosphate molecule (GDP). The GDP-bound heterotrimeric state is a stable entity. After ligand-receptor interaction, conformational changes within the receptor cause the G-protein to exchange GDP for GTP (guanosine triphosphate). The GTP activates the G-protein, causing dissociation of α -sub-unit, which binds to and activates the intracellular effector. The α -sub-unit hydrolyses GTP to GDP, and then

successfully re-associates with the β - and γ -sub-units. Stimulation of tachykinin receptors initiates the cascade of phosphoinositol in many tissues, including the salivary gland and brain in rats and tracheal smooth muscle in guinea pigs (Mantyh *et al.*, 1984; Hunter *et al.*, 1985; Grandordy *et al.*, 1988) leading to the production of inositol 1,4,5- trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ releases intracellular calcium acting on specific receptors in the sarcoplasmic reticulum. DAG increases intracellular calcium by opening voltage-gated Ca²⁺ channels via protein kinase C (PKC) (Gallacher *et al.*, 1990). Although PLC was considered to be the principal intracellular effector for tachykinin receptors, in certain cells, tachykinin receptor activation might be coupled to adenylyl cyclase activation (probably via G_s) in cultured neuroblastoma cells and thyroid gland in dogs, or to adenylyl cyclase inhibition (probably via G_i) in rat salivary gland (Narumi and Maki, 1978; Yamashita *et al.*, 1983; Laniyonu *et al.*, 1988). Thus depending on the cell type, tachykinins elicit their effects by activating multiple effectors via different G-proteins.

Some studies have shown that the activation of NK-2R and NK-1R by SP and NKA is mediated by the release of arachidonic acid (AA) and the production of prostaglandin E₂ (Catalioto *et al.*, 1998).

The activation of NK-1R may modulate ion channels. For example, NK-1R stimulation could induce the opening of chloride channels in smooth muscle cells of rabbit colon or inhibit K⁺ channel inward and L-type calcium channels in submandibular ganglion neurons in hamsters (Nakajima *et al.*, 1988).

The substitution of the amino acid sequence located at the third loop of intracellular NK-1R by an equivalent sequence from the NK-2R did not change the affinity of SP to its receptor, but avoided the generation of second messengers IP_3 and cAMP. This suggested that the third intracellular loop of NK-1R is particularly important for the activation of intracellular second messengers (Blount and Krause, 1993).

1.7 Tachykinins in cardiovascular regulation

The possible site of action for the cardiovascular response associated with the defense reaction in rats is the hypothalamus (Culman and Unger, 1995). The paraventricular, ventromedial and dorsomedial hypothalamic regions are parts of the CNS rich in SP (Brownstein *et al.*, 1976). The hypothalamus is an area that is particularly important in the autonomic control of the cardiovascular system. Moreover, SP that is injected into the anterior and ventromedial hypothalamus induced a defense reaction response that is very similar to that observed following its i.c.v. injection (Itoi *et al.*, 1991, 1994). Tachykinins and their receptors are also found in other CNS parts recognized in cardiovascular regulation. SP and their binding sites are present in the medulla oblongata (Douglas *et al.*, 1982; Nevin *et al.*, 1994) and in the NTS (Helke *et al.*, 1980). Data also suggested a role for SP in regulating the baroreflex (Helke *et al.*, 2004). I.c.v. injection of SP or phosphoramidon, an inhibitor of endopeptidases, improved the sensitivity of the baroreflex, while antibodies against SP injected in the cerebral ventricles or directly into the NTS reduced baroreceptor reflex (Chan *et al.*,

1990; Appenrodt *et al.*, 1993). Moreover, SP or NK-1R agonist injected into the NTS induced bradycardia and hypotension (Kubo and Kihara, 1987; Feldman, 1995).

Tachykinin NK-1R, NK-2R and NK-3R agonists injected into the i.c.v. increased BP and HR together with stereotyped behaviours in rats (Itoi *et al.*, 1992; Picard *et al.*, 1994; Cellier *et al.*, 1997). Cardiovascular effects to i.c.v. injected NK-3R agonists depended on the activation of the sympathetic nervous system, and also on the release of vasopressin from the hypothalamus, since the increase of BP induced by NK-3R activation was blocked by vasopressin V1 antagonist (Unger *et al.*, 1981, 1988; Polidori *et al.*, 1989; Takano *et al.*, 1990; Cellier *et al.*, 1997). In SHR, the content of NKB in the supraoptic nucleus of the hypothalamus and in the NTS was higher than in normotensive rats (Nagashima *et al.*, 1989). Furthermore, the tonic activation of NK-3R by endogenous tachykinins in the SN of SHR contributed to maintain high BP, since the intra-nigral administration of a NK-3R antagonist significantly decreased MAP (Lessard *et al.*, 2003). Moreover, tachykinin agonists injected into the SN, an important DA pathway, induced tachycardia that was blocked by selective tachykinin antagonists (Lessard and Couture, 2001). However, the neuronal pathways involved in cardiovascular and behavioural effects induced by tachykinins following i.c.v. administration are still not fully known.

NK-1R binding sites in the spinal intermediolateral horn were located on dendrites and cell bodies of sympathetic pre-ganglionic neurons (Charlton

and Helke, 1987). The intrathecal (i.t.) injection of agonists in T8-T10 of freely moving rats was used to evaluate the effect of tachykinins on these neurons. The i.t. injection of SP and NK-1R agonists induced increases in BP and HR and these cardiovascular effects were accompanied by the release of norepinephrine and epinephrine (Hassessian *et al.*, 1990; Couture *et al.*, 1995).

The role of NK-2R and NK-3R in cardiovascular regulation at the level of the spinal cord is uncertain. However, NK-2R were located on astrocytes at the edge of the dorsal and ventral horns and around the central canal, while NK-3R immunoreactivity were situated in superficial layers (laminae I and II) of the dorsal horn and laminae X in rat spinal cord, suggesting that some effects of tachykinins on somatosensation may be mediated by NK-3R, and that NK-3R may modulate primary afferent, sensory information, both post- and pre-synaptically (Zerari *et al.*, 1998; Seybold *et al.*, 1997; Couture *et al.*, 2000). Furthermore, in 2006, Cloutier *et al.* provided the first functional and autoradiographic evidence that NK-1R and NK-3R are up-regulated in the spinal cord of SHR. The study showed hypersensitivity of the pressor and tachycardiac responses induced by i.t. injection of NK-1R and NK-3R agonists in SHR. This suggested that the spinal tachykinergic system contributes to the hyper-sympathetic activity in SHR.

1.8 Tachykinins in spinal nociception

NK-1R and NK-2R agonists injected i.t. decreased the reaction time to a painful thermal stimulus in rats and, therefore, induces hyperalgesia (Laneuville *et al.*, 1988; Picard *et al.*, 1993; Severini *et al.*, 2002). The role of NK-3R in the process of pain was less clear. Indeed, some studies reported that activation of NK-3R could have induced hyperalgesia through the release of nitric oxide (NO) (Linden *et al.*, 1999), particularly during peripheral inflammation (Ackley *et al.*, 2001), while other studies reported analgesia via the local release of opiates following intrathecal injection of NK-3R agonists (Laneuville *et al.*, 1988; Couture *et al.*, 2000).

1.9 Tachykinins in behavioural activity

Tachykinin receptors localized in brain regions were implicated in affective behaviour and in the adaptation responses to stress in rats and mice (Ebner and Singewald, 2006; Yan *et al.*, 2009). Central injection of tachykinins induced behavioural activity in rats. Injection into the cerebral ventricles of NK-1R and NK-2R agonists increased locomotion, grooming and sniffing, while the injection of NK-3R agonists induced mostly wet-dog shake behaviour (Tschöpe *et al.*, 1992; Itoi *et al.*, 1992; Picard *et al.*, 1994; Cellier *et al.*, 1997, 1999).

Behaviours induced by tachykinins such as grooming and locomotion were also observed in affective disorders such as addiction and opiate withdrawal, depression, anxiety, and stress in guinea pigs and rats. In fact, SP is the main tachykinin involved in these disorders (Kramer *et al.*, 1998;

Murtra *et al.*, 2000; Gavioli *et al.*, 2002). The induction of acute stress in rats affected the concentration of SP and the density of NK-1R in several regions of CNS including the limbic system (Takayama *et al.*, 1986; Siegel *et al.*, 1987; Rosén *et al.*, 1992). NKA, the endogenous ligand for NK-2R, coexisted with SP within the same neuronal population and was co-released with the latter peptide under stressful stimulus (Griebel *et al.*, 2001; Steinberg *et al.*, 2001).

SP injected in the i.c.v. of conscious rats caused a typical defense reaction consisting of a behavioural response associated with activation of the cardiovascular system. This reaction was characterized by an increase in locomotion, grooming, scratching and biting at the skin. These behaviours were accompanied by an increase of sympatho-adrenal activity, BP, HR, renal and mesenteric vasoconstriction and vasodilatation of skeletal muscle in the hind limbs (Unger *et al.*, 1988; Culman and Unger, 1995).

2. The ventral tegmental area

2.1 Location in the CNS

The ventral tegmental area (VTA) in the ventral midbrain constituted a significant portion of the extrapyramidal system of the basal ganglia that was medial to the SN and ventral to the red nucleus (Fields *et al.*, 2007) (Figure 4). The VTA and SN pars compacta were the sole sources of striatal and limbic forebrain DA. These pathways made projections into the nucleus accumbens of the striatum in the pre-frontal cortex and the limbic system, including the septum and amygdala (Otsuka and Yoshioka, 1993). In rats, the VTA was divided mainly in two parts: a region called the dorsal nucleus pigmented para-pharyngeal, and the caudal ventrolateral region called the nucleus para-nigral (Halliday and Tork, 1986; Fallon and Loughlin, 1995). From a morphological point of view, the VTA was characterized by a majority of fusiform neurons, small to medium size and rather oriented on a horizontal plane (Swanson, 1982).

2.2 Roles of the VTA

The VTA originating in the midbrain is connected to the limbic system and cortex which are two systems typically involved in emotions, motivation and motor functions. Moreover, the VTA has a large number of afferent and efferent projections and is also the center of the network of the neural pathways involved in several major functions of the CNS.

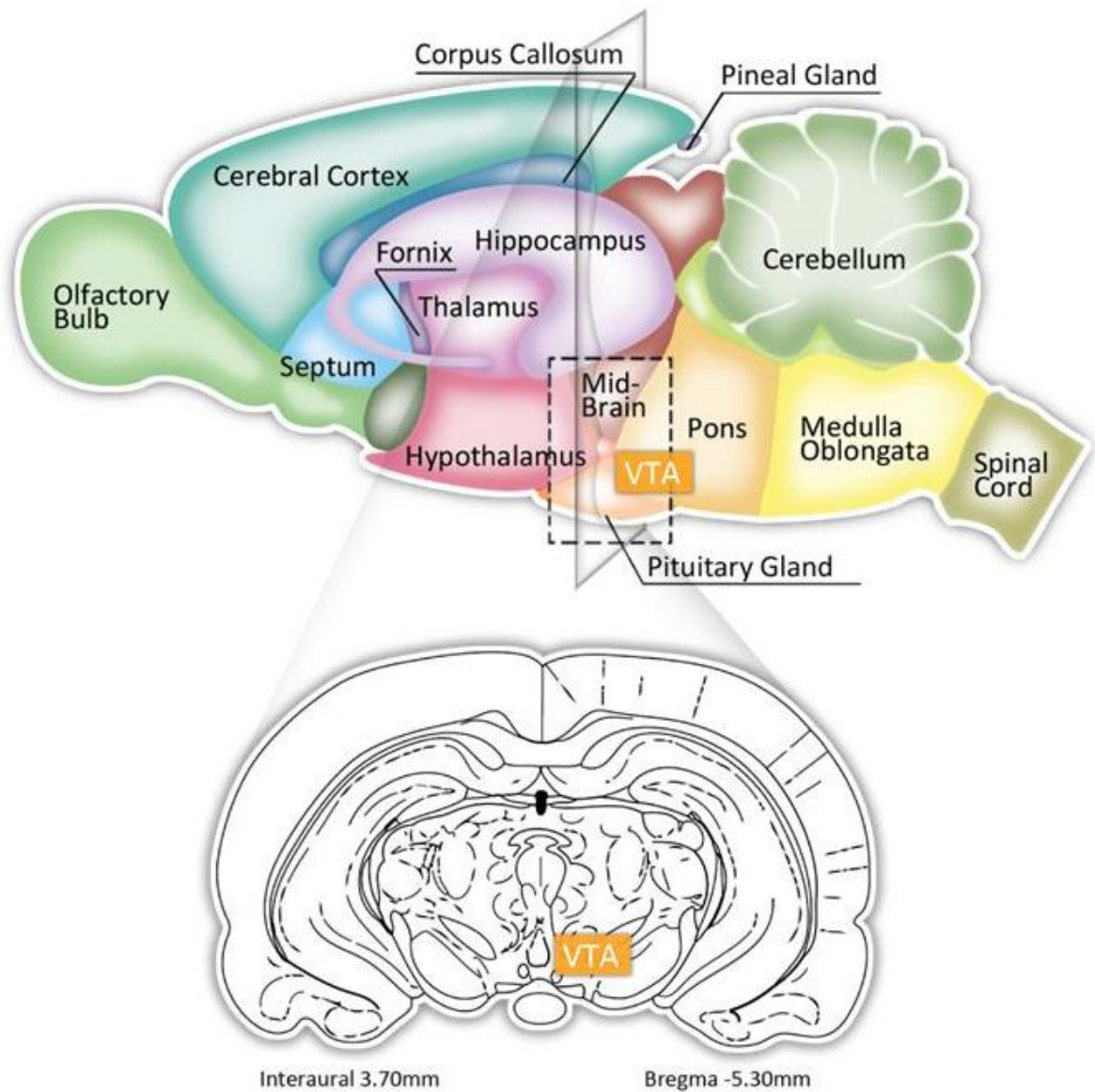


Figure 4. Location of the VTA in the rat brain.

2.2.1 Neuronal efferent and afferent of the VTA

2.2.1.1 Major efferents

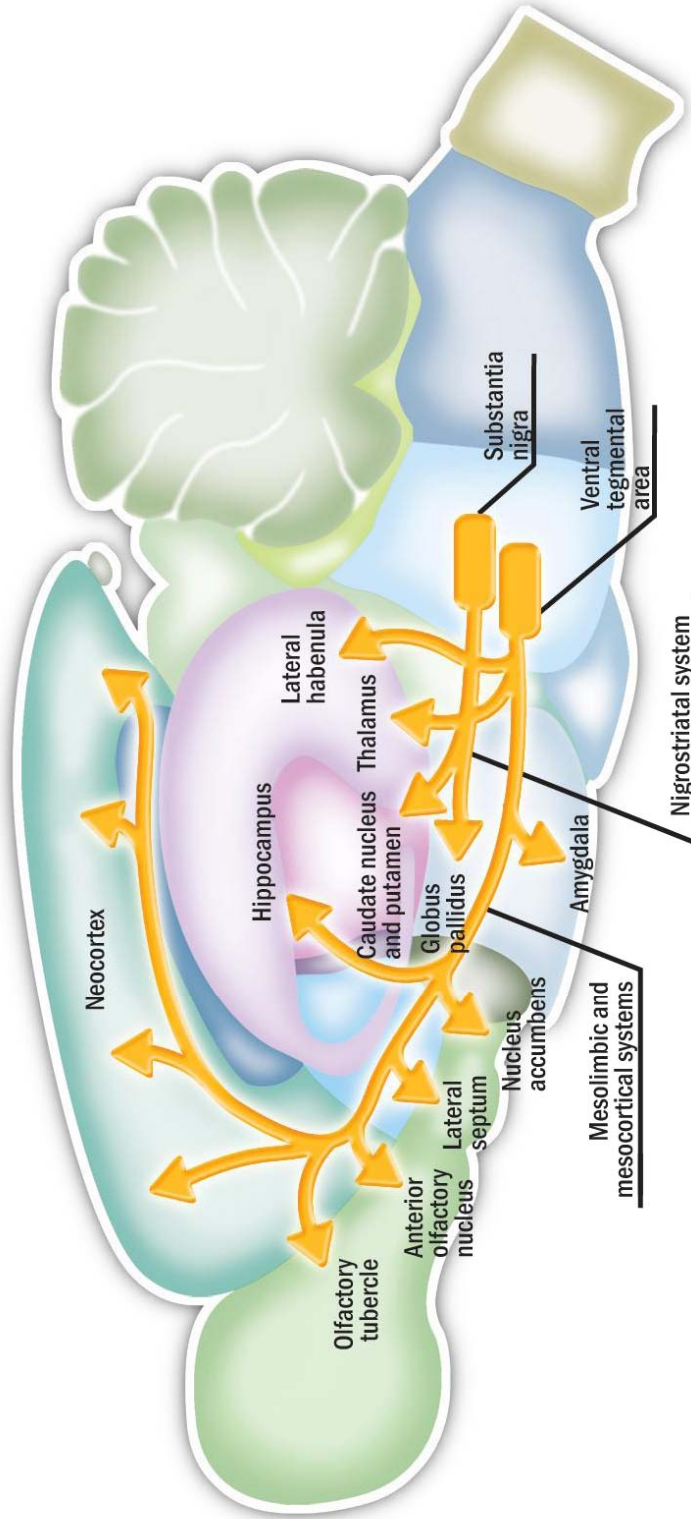
The VTA system with its mesocorticolimbic projections can be broadly divided in two projections: mesolimbic and mesocortical projections (Figure 5). The mesolimbic projections include efferents to the nucleus accumbens (ventral striatum), olfactory tubercles, the central nucleus, medial and lateral amygdala, the septal region that includes the basal nucleus of the stria terminalis and lateral septum, the hippocampus, the middle part of the thalamus (lateral and medial habenula) and supraoptic nucleus of the hypothalamus. Mesocortical projections include efferents to the prefrontal cortex, entorhinal, cingulate and occipital (Figure 5). In addition, the VTA has projections to the periaqueductal gray, the parabrachial nucleus, the locus coeruleus and the dorsal and medial raphe nucleus, which showed implications in the control of the autonomic nervous system (Swanson, 1982; Oades and Halliday, 1987; Pierce and Kumaresan, 2006).

Three less important pathways also exist: the mesostriatal, the mesodiencephalic, and the mesorhombencephalic pathways. Below is a brief summary of where each pathway originates and terminates:

- ✓ Mesostriatal: originates in the SN and VTA; innervates the anteromedial striatum.
- ✓ Mesodiencephalic: originates in the SN and VTA; innervates several thalamic and hypothalamic nuclei.

- ✓ Mesorhombencephalic: originates in the SN and VTA; innervates the monoaminergic nuclei, superior colliculus and periaqueductal gray.

Figure 5. Schematic midsagittal section of a rat brain, showing the locations of the most important groups of dopaminergic neurons and the distribution of their axons and terminal buttons.



Adapted from Chapman and Hall, 1993.

2.2.1.2 Major afferents

The VTA received many afferents from different regions of the CNS (Phillipson, 1979; Geisler and Zahm, 2005) including afferents from: the amygdala (Wallace *et al.*, 1992), the nucleus accumbens (Heimer *et al.*, 1991), the prefrontal cortex (Sesack and Pickel, 1992), the dorsal raphe nucleus (Gervais and Rouillard, 2000), the medial habenular nucleus (Cuello *et al.*, 1978) and the lateral preoptic area and medial hypothalamus (Swanson, 1982). The chemical nature of these afferents was diverse. For example, a projection of the "shell" of the nucleus accumbens core paraventricular VTA involved enkephalinergic and GABAergic fibers (Kalivas *et al.*, 1993). The afferents of the prefrontal cortex synapses were on the dendrites of DA neurons and GABAergic neurons (Carr and Sesack, 2000). In addition, the afferents of the glutamatergic pedunculopontine nucleus was shown to make synaptic contacts with the midbrain DA neurons in squirrels and monkeys (Smith *et al.*, 1996). The afferents of the serotonergic dorsal raphe nucleus may have synapses on DA and non-DA neurons (Herve *et al.*, 1987; Van Bockstaele *et al.*, 1994).

2.3 Main receptors and neurotransmitters found in the VTA

The VTA area was composed mainly (80%) of DA neurons. This group of neurons forms the A10 area. Neurons with hydroxylase-labeled tyrosine are found in all VTA (Swanson, 1982; van de Buuse and Catanzariti, 2000). Autoradiography studies have reported that D2R located in the dorsal part of the VTA are localized on the soma and dendrites of DA neurons, and

thus play a role as auto-receptors (Bouthenet *et al.*, 1987; Sales *et al.*, 1989; Chen and Pan, 2000). However, there was also D2R at presynaptic terminals on neuronal afferent (Pickel *et al.*, 2002). Moreover, the D1R was presented in the VTA (Dubois *et al.*, 1986). These receptors were located on terminals of GABAergic and glutamatergic afferents innervating the VTA (Smith *et al.*, 1996; Steffensen *et al.*, 1998; Ranaldi and Wise, 2001). Thus, unlike the D2R, the D1R may modulate indirectly DA neurons, and rather by controlling the release of neurotransmitters in afferents innervating the VTA. Electrophysiological and immunocytochemistry studies showed the presence of non-DA neurons containing GABA in the VTA (Steffensen *et al.*, 1998; Carr and Sesack, 2000). A certain portion of these cells was made of local inter-neurons providing inhibitor "inputs" to DA neurons (O'Brien and White, 1987; Johnson and North, 1992b; Steffensen *et al.*, 1998).

Neuroanatomical and functional studies suggested the presence of GABA_A and GABA_B receptors in the VTA, which were densely distributed in the paranigral region (Churchill *et al.*, 1992; Rodriguez *et al.*, 2000). Moreover, it was also reported the existence of a large network of GABAergic neurons in the VTA that are interconnected via gap junctions, which have projections to the nucleus accumbens and prefrontal cortex (Van Bockstaele and Pickel, 1995; Carr and Sesack, 2000). This network allows for electrical conduction, which is considerably faster than the chemical conduction of signals between synapses (Olson and Nestler, 2007). Among other neurotransmitters and receptors, immunofluorescence studies identified a subpopulation of DA neurons containing neurotensin (NT) and

cholecystokinin in the VTA (Seroogy *et al.*, 1987, 1988). The receptors for NT are presynaptic and somatodendritic on DA neurons (Legault *et al.*, 2002). Several studies also suggested the presence of nicotinic, serotonin (5-HT₂), NMDA, and metabotropic glutamate receptors on DA neurons, and Mu opioid receptors on GABAergic interneurons in the VTA (Doherty and Pickel, 2000; Klink *et al.*, 2001; Zheng and Johnson, 2003; Swanson and Kalivas, 2000; Bergevin *et al.*, 2002).

2.4 Location and function of tachykinins in the VTA

The injection of SP, NKA and the analogue of SP DiMe-C7 into the VTA in rat caused an increase in episodes of physical shock, exploration and locomotion (Elliott and Iversen, 1986). The VTA releases SP and it was reported that its levels decline after electric shock in rats. However, the NKA levels are not altered by this manipulation (Bannon *et al.*, 1986). It is interesting to note that stress conditions activate the DA pathway A10 whose origin lies in the VTA without affecting the pathway A9, which originates in the SN (Bannon *et al.*, 1983, 1986). The tachykinins in the VTA modulate the activity of DA A10 since DA levels in the nucleus accumbens and the prefrontal cortex were increased after senktide injected into the VTA in guinea pig (Marco *et al.*, 1998). These effects were completely inhibited by the addition of a selective antagonist of NK-3R, SR142801 (Marco *et al.*, 1998). Moreover, addition of senktide or NKB on rat VTA slices caused a local DA increase (Seabrook *et al.*, 1995). Endogenous tachykinins released in the VTA activate both the tachykinin NK-1R and

NK-3R. Several studies by autoradiography, immunohistochemistry and electron-microscopy showed the presence of NK-3R in the VTA (Smith and Flynn, 2000; Langlois *et al.*, 2001; Lessard *et al.*, 2003, 2007).

Several behaviours were induced following injection of NK-1R, NK-2R and NK-3R agonists in specific nuclei of the CNS such as the SN, VTA and median raphe nucleus. Behaviours induced by NK-1R agonist [Sar⁹, Met (O₂)¹¹]SP, NK-2R [β -Ala⁸]NKA (4-10) and NK-3R agonist senktide depended on central dopaminergic activation in the SN and VTA (Stoessl *et al.*, 1991; Elliott *et al.*, 1992; Mason and Elliott, 1992; Overton *et al.*, 1992; Lessard *et al.*, 2003; Deschamps and Couture 2005). This was consistent with the presence of endogenous tachykinins and NK-3R in the VTA (Langlois *et al.*, 2001). The increase in locomotor activity induced by NK-1R agonists in the VTA was increased by the peripheral administration of D-amphetamine, a drug known to stimulate the release of DA and inhibiting its reuptake (Eison *et al.*, 1982). In contrast, this behaviour was inhibited by haloperidol, a non-selective DA antagonist with high affinity for the D2R (Miyamoto *et al.*, 2005) and for sigma 1 and sigma 2 receptors (Hashimoto and Ishiwata, 2006). Haloperidol exerts lower affinity for DA-D1R, D3R or D4R, serotonin 2A receptor, and α 1 adrenergic receptor (Miyamoto *et al.*, 2005). The locomotor activity induced by NK-1R agonist in the VTA was also blocked by a lesion in the A10 DA neurons (Takano *et al.*, 1985; Elliott *et al.*, 1992). The wet-dog shakes induced by the activation of NK-3R were however dependent on endogenous serotonin. Indeed, wet-dog shakes

induced by senktide injected i.c.v. in rats were blocked by antagonists of serotonin 5-HT₂ and 5-HT_{1c/2}. Another argument suggesting the involvement of endogenous serotonin was the cause of intensification of behaviour in the presence of an inhibitor of serotonin reuptake (Stoessl *et al.*, 1988, 1990).

3. Centrally acting anti-hypertensive agents

Most of the traditional drugs used to reduce elevated blood pressure interact with various elements of the sympathetic nervous system (SNS): in fact, almost every component of this system can be modulated by drugs (Noll *et al.*, 1998). In addition, diuretics and various types of vasodilators have been introduced into anti-hypertensive therapy, and the renin-angiotensin-aldosterone system has been recognized as an important target of anti-hypertensive therapeutics. Ganglionic blocking agents, postganglionic blocking agents (guanethidine), and reserpine owed their anti-hypertensive potency to their interaction with particular structures of the SNS (Plummer and Yonkman 1960). Despite their anti-hypertensive efficacy, they are no longer acceptable for chronic treatment due to their multiple and severe side effects. The same holds for "directly" acting vasodilators such as hydralazine and minoxidil, which cannot be used in monotherapy. At present, vasodilation is preferably induced by newer drugs, such as calcium antagonists, ACE-inhibitors or selective α_1 -adrenoceptor antagonists (van Zwieten, 2002).

Central sympatholytic cardiovascular agents reduce the sympathetic nerve output and are more effective than β -blockers. A "centrally acting" sympatholytic cardiovascular agent is one that when administered by a conventional route, oral or i.v., is capable of crossing the blood-brain barrier and can produce effects in the CNS. Suitable central acting agents like adrenergic stimulants include methyldopa, clonidine, and guanfacine. It is also known that certain local anesthetics such as lidocaine, bupivacaine, and ropivacaine when administered directly to a sympathetic nerve, produce a local effect on the neurons that function in a sympatholytic manner (Hildebrand *et al.*, 2005).

The sympatholytic cardiovascular agent is preferably delivered into the CNS which provides access at the sub-arachnoid space of the thoracic spinal cord between the first and fifth thoracic vertebrae; the sympathetic preganglionic cell bodies located in the intermediolateral cell column of the spinal cord; the preganglionic sympathetic neurons which provide innervation to the heart; and the preganglionic sympathetic neurons which provide innervation to the kidneys to prevent stimulation of the renin-angiotensin system (Hildebrand *et al.*, 2005).

Clonidine and methyldopa were the examples of central acting α_2 -adrenoceptor agonists (Amery *et al.*, 1970). Several studies have, however, reported the involvement of central imidazoline receptors in the central anti-hypertensive effect of these drugs (van Zwieten, 1995). Due to their adverse reactions, these effective anti-hypertensive drugs had lost their importance. Several of these adverse reactions, in particular sedation and

the occurrence of a dry mouth, were mediated by central α_2 -adrenoceptors (Kaufman, 1989). Rilmenidine and moxonidine were examples of drugs, which activated the central imidazoline receptors and consequently, reduced hypertension. Their effect on α_2 -adrenoceptors was weak and these compounds have been claimed to cause less sedation than clonidine and methyldopa (Bock *et al.*, 1999). This gives a possibility to develop centrally acting anti-hypertensive drugs which cause less sedation than the classical drugs (Bricca *et al.*, 1989; Chrisp and Faulds, 1992).

3.1 Clonidine

Clonidine is a α_2 -adrenergic receptor agonist, which selectively stimulates postsynaptic α -adrenergic receptors in the depressor site of the vasomotor center of the medulla oblongata in the region of the NTS and locus coeruleus (Kosman, 1975; Svensson *et al.*, 1975). The activation of these central α -adrenergic receptors reduced the efferent sympathetic neuronal vasoconstrictor tone to the heart, kidneys, and peripheral vasculature causing vasodilatation and lowering blood pressure (Hausler, 1982; Rockhold and Caldwell, 1980). Multiple sites within the CNS were sensitive to clonidine (Hausler, 1982). The rostral ventrolateral medulla (RVLM), an area of the medullary reticular formation that contains neurons projecting to the spinal cord (Reis *et al.*, 1988), is the termination site of afferent baroreceptor neurons responsible for the control of blood pressure, which is an important target for clonidine-induced hypotension (Schreihofer and Guyenet, 2000). Clonidine can also reduce stimulation-

evoked release of noradrenaline from various isolated tissues through an action at prejunctional inhibitory α_2 -adrenoceptors (Starke *et al.*, 1977).

3.2 Guanabenz

Guanabenz produced its hypotensive effect, at least in part, by means of a CNS clonidine-like sympatho-inhibition. However, the precise extent of central sympatho-inhibitory action has not been established. For example, guanabenz possesses pharmacological actions similar to both clonidine and guanethidine and consequently also reduced blood pressure and heart rate by peripheral pre- and postsynaptic anti-adrenergic effects (Koss, 1983).

3.3 Moxonidine

Moxonidine is a selective agonist acting at imidazoline I₁ receptors with only minor activity at α_2 -adrenoceptors. It exerts its anti-hypertensive effect by an action in the CNS reducing peripheral sympathetic activity and decreasing peripheral vascular resistance. The site of action of moxonidine in the CNS is thought to be the RVLM (Haxhiu *et al.*, 1994).

3.4 Methyldopa

Methyldopa is an analog of DOPA (3,4-hydroxyphenylalanine) and a pro-drug which must be converted to an active metabolite in order to exert its effects in the CNS. The decrease in blood pressure caused by methyldopa was discovered in hypertensive human over five decades ago (Oates *et al.*, 1960; Mah *et al.*, 2009). In the 1970's and 80's methyldopa was considered to be an effective anti-hypertensive agent especially in the elderly and patients with renal insufficiency, and pregnant women. Methyldopa exerts

a hypotensive effect by interfering with the synthesis and action of noradrenaline. It inhibits the conversion of dopa into dopamine by competing with the enzyme dopa decarboxylase and thus reduces the amount of noradrenaline formed from dopamine (Vaidya *et al.*, 1970).

Later on, methyldopa was recommended as adjunct therapy after diuretics (Goodman and Gilman, 1996). It has since been associated with a wide spectrum of adverse events including CNS depressant effects such as drowsiness, fatigue, lethargy, depression, decreased libido, dry mouth, hepatitis, myocarditis, and haemolytic anaemia (Hoffman, 2006; Mah *et al.*, 2009).

4. Dopamine

DA is one of the main catecholamines in mammals. It plays an important role as a brain neurotransmitter and is well known for its contribution to the development of pathologies, mainly in arterial hypertension (Contreras *et al.*, 2002). Moreover, a vast variety of psychological functions, such as motor behaviour, cognition, emotion, food intake were related to DA. Parkinson's disease, schizophrenia, drug addiction, attention deficit hyperactivity disorder (ADHD) and depression, which are severely disabling neurological diseases, are also associated with dysfunctions in the DA system (Liss and Roeper, 2008; Altar *et al.*, 2009). Within the CNS, DA binds to specific membrane receptors presented by neurons, and it played the key role in the control of locomotion, learning, working memory, cognition, and emotion (Missale *et al.*, 1998).

The first evidence for the existence of DA receptors in the CNS came in 1972 from biochemical studies showing that DA could stimulate adenylyl cyclase (AC). This observation came the suggestion to call the receptors: D1 which stimulated AC, and D2 the one that was not coupled to this effect (Kebabian and Calne, 1979).

Analysis of the primary structure of the cloned DA receptors revealed that they were members of the seven transmembrane domain GPCR family and shared most of their structural characteristics. Members of this family displayed considerable amino acid sequence conservation within seven transmembrane domains (for review, see Missale *et al.*, 1998).

4.1 Dopamine receptors

Pharmacological, biochemical, anatomical, and physiological studies have indicated that the D1, D2, D3, D4 and D5 are receptor subtypes for DA (Creese *et al.*, 1982; Stoof *et al.*, 1984; Lebel *et al.*, 2007). Physiological functions of the brain DA system were well recognized. However, DA biosynthesis did not only occur in neurons, but also in peripheral tissues. Moreover, studies *in situ* hybridization revealed high concentrations of D2R mRNA in the neostriatum, olfactory tubercle, SN, VTA and nucleus accumbens (Meador-Woodruff *et al.*, 1991, 1992). The striatum contains DA terminals, which received information from the VTA; 80% of the cells in this structure were medium spiny neurons (MSN) (Coskran *et al.*, 2006; Seeger *et al.*, 2003) containing DA-D1R or DAR receptors. The D3R expression was similar to D2R, although with much lower levels in several

brain areas. D3R was found in limbic areas such as nucleus accumbens, the islands of Calleja and dentate gyrus of the hippocampus (Suzuki *et al.*, 1998; Bouthenet *et al.*, 1991).

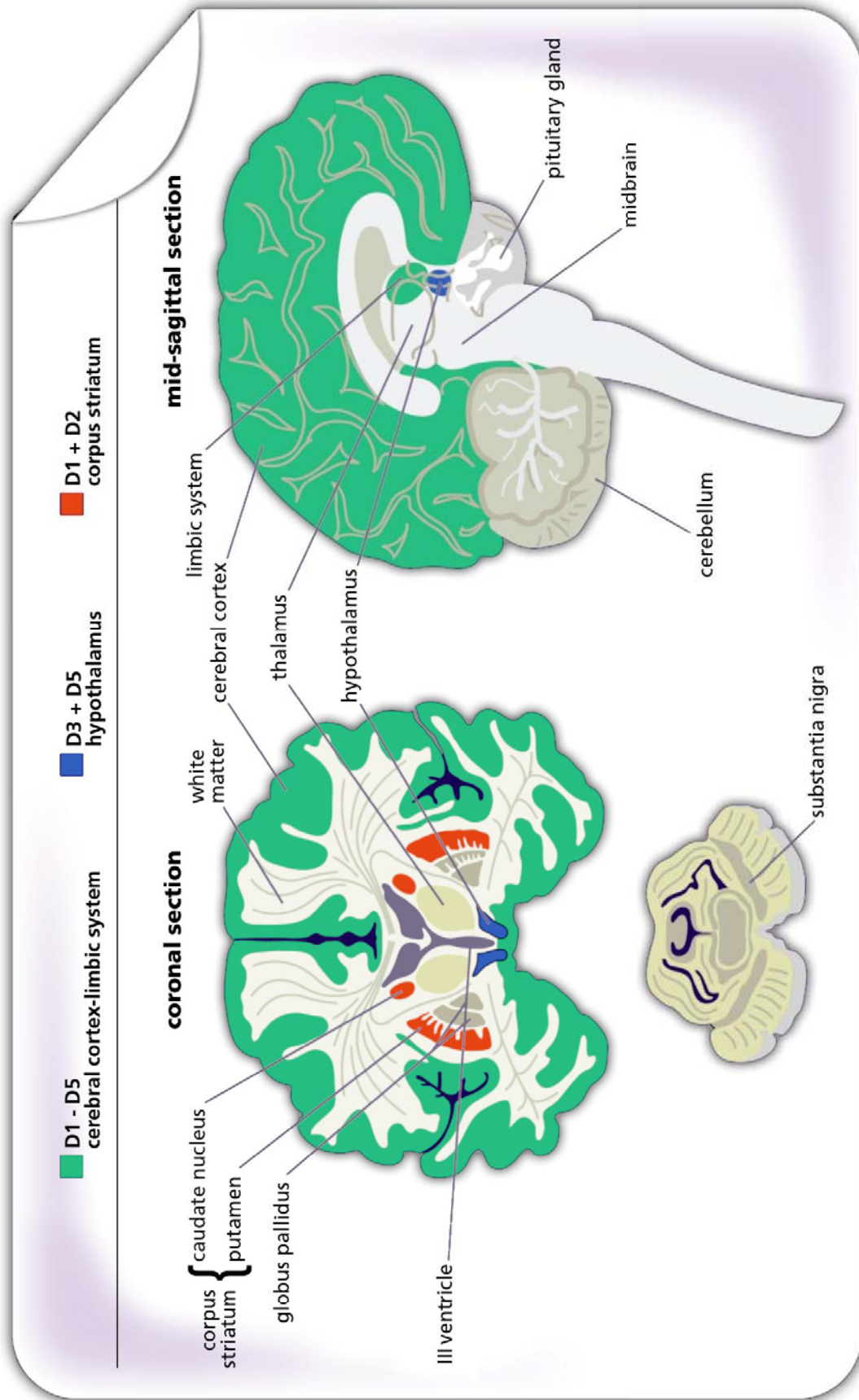
The D4R had more restricted distribution in the rat brain with a little expression in the nucleus accumbens and caudate putamen, although D4R was expressed in this rank order: hippocampus > caudate putamen > olfactory tubercle = SN > nucleus accumbens core > cerebral cortex > cerebellum (Lahti *et al.*, 1995; Jackson *et al.*, 1994; Defagot and Antonelli, 1997).

Studies *in situ* hybridization mRNA were performed to determine the distribution of D5R in rats. However, only two structures were identified as containing D5R mRNA: the hippocampus, and the perifornical nucleus of the thalamus. This mRNA could not be visualized in the more traditional brain regions related with DA cell bodies or projection fields, proposing a different function in the brain for D5R (Meador-Woodruff *et al.*, 1992).

Most antipsychotics (psychiatric medications primarily used to manage psychosis, including delusions or hallucinations) were developed as D2R antagonists. They were likely to bind to the D2R with higher affinity than to the D3R and D4R. Conversely, a few antipsychotics have been established to show selectivity for the D3R or D4R. Through these, some aspects of the functions of the D3R and D4R may be revealed. Clozapine, an atypical antipsychotic whose actions are not accompanied by adverse motor side effects, shows a higher selectivity for the D4R than for any other

D2R-like. Later, it was shown that clozapine effect on D1R and D2R, may have been responsible for its exclusive efficacy in schizophrenia patients (Weiner *et al.*, 1991; Tauscher *et al.*, 2004) (For DAR distribution in the CNS see Figure 6).

Figure 6. Schematic representation of the DA receptors in the human brain.



Adapted from Rang and Dale, 2001.

Several DA antagonists have been discovered such as SCH23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol], which is a selective DA-D1R antagonist, and raclopride (3,5-dichloro-N-[[[(2S)-1-ethyl-2-pyrrolidinyl]methyl]-2-hydroxy-6-methoxybenzamide), which is a selective DA-D2R antagonist. Both antagonists had pharmacologic effects which were similar to standard antipsychotics (Iorio *et al.*, 1983; Hall *et al.*, 1989).

4.2 Dopamine: molecular signaling pathways

Five subtypes of mammalian DAR were grouped into two classes, with the D1-like receptor, composed of the D1R and D5R subtypes, and the D2-like receptor, composed of the D2R, D3R, and D4R subtypes. There was also some evidence suggesting the existence of possible DA-D6R and D7R, but such receptors have not been conclusively identified (Contreras *et al.*, 2002).

All of the DAR are G protein-coupled receptors (GPCRs), whose signaling is primarily mediated by interaction with an activation of heterotrimeric GTP-binding proteins (G proteins). Members of this superfamily were called 7-transmembrane receptors, since they traverse the cell membrane seven times (Neves *et al.*, 2002).

The activation of D1R-like is coupled to the G-protein $G_{\alpha s}$, which subsequently activates adenylyl cyclase, raising the intracellular concentration of the second messenger cyclic adenosine monophosphate (cAMP). Activation of D2R-like family is coupled to the G-protein $G_{\alpha i}$,

which directly inhibits the formation of cAMP by inhibiting the enzyme adenylate cyclase (Neves *et al.*, 2002) (Figure 7).

In the brain, cAMP levels have been implicated in genetic transcription processes (Lalli and Sassone-Corsi, 1996), ionic channel activation, DA neuron survival (Yamashita *et al.*, 1997), synaptic plasticity (Zhong and Wu, 1991), neurotransmitter release (Bouron and Reuter, 1999), locomotion, sensory perception and in memory and learning processes (Frey *et al.*, 1993; Tully *et al.*, 2003). In striatal cells, the increase in cAMP levels leads to activation of protein kinase A (PKA), which plays a role in reward mechanisms of drug consumption, locomotion activation and anxiety (Kim *et al.*, 2008). Striatal cells, integrators of DA signaling from VTA, SN, cortical and thalamic afferent messages, were capable of regulating motor, sensory or cognitive action patterns. Thus, the control of the cAMP/PKA pathway was very strict and its impairment could be responsible for the development of psychotic processes, neurological alterations such as Parkinson's and Alzheimer's diseases and anxiety-related disorders. Adenylyl cyclase activities and expression have been identified in striatum (AC1, AC5, AC8, and AC9), where AC5 is the most abundant in that brain region (Glatt and Snyder, 1993; Matsuoka *et al.*, 1997).

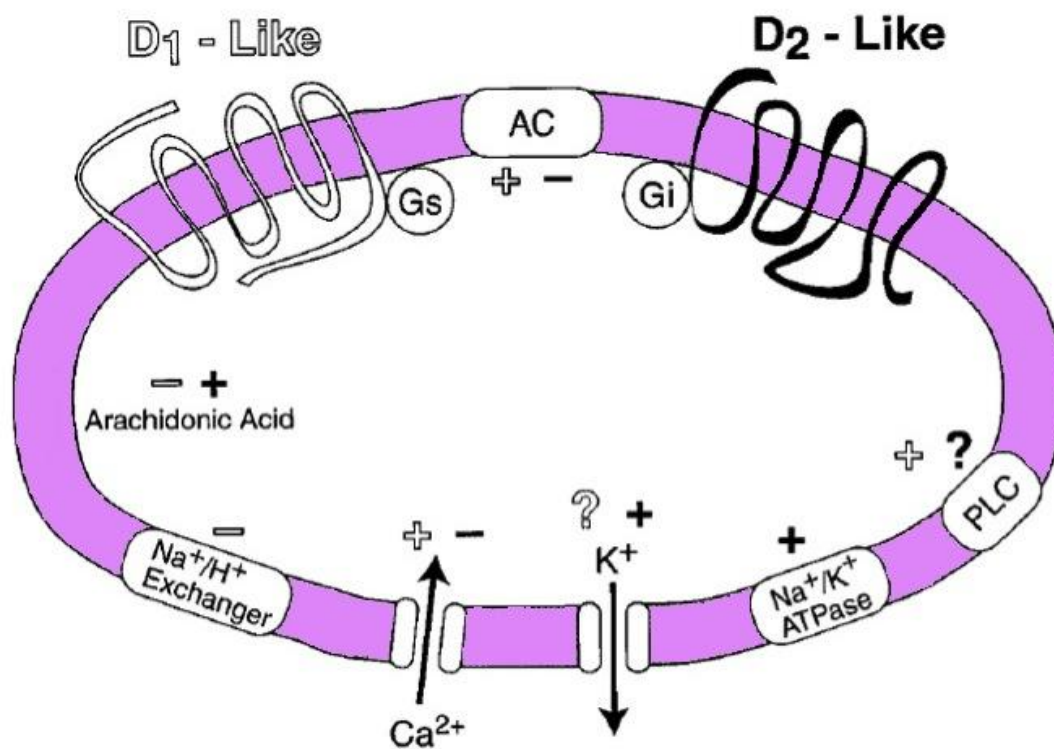


Figure 7. Signal transduction of D1R-like and D2R-like receptors. AC, adenylate cyclase; PLC, phospholipase C. Adapted from Missale et al., 1998.

4.3 Dopamine in cardiovascular regulation

The brain dopaminergic system (mainly the nigrostriatal pathway) which connects the SN with the striatum, is one of the four major DA pathways in the brain. It is particularly involved in the production of movement, as part of a system called the basal ganglia. There are several pharmacological studies *in vivo* supporting an involvement of this central DA system in cardiovascular regulation (van den Buuse, 1986; van den Buuse and Catanzariti, 2000).

Chemical or electrolytic lesions of the nigrostriatal DA system in SHR during the prehypertensive stage attenuate the development of hypertension (van den Buuse *et al.*, 1986, Linthorst *et al.*, 1994). Moreover, elevated tyrosine hydroxylase activity and higher dihydroxyphenylacetic acid (DOPAC) concentrations have been reported in the striatum of SHR. These results suggested that the nigrostriatal DA system in SHR was hyperactive and this hyperactivity caused the development of hypertension (Nagaoka and Lovenberg, 1994; Linthorst *et al.*, 1994).

In recent years, along with other observations in rat models of hypertension, SHR, DOCA-salt, and in patients with an impaired central DA system, the VTA was given a role in central cardiovascular regulation. This mesolimbic DA system plays a role in the regulation of diurnal blood pressure and heart rate in rats. Normally, during sleep, blood pressure decreases. However, a lesion of the mesolimbic DA system due to 6-hydroxydopamine (6-OHDA) injected into the VTA inhibited the decrease in blood pressure in rats during their resting period (Sakata *et al.*, 2002).

I.c.v. injections of 6-OHDA in young SHR inhibited the subsequent development of hypertension (van den Buuse *et al.*, 1986). This suggested that the central DA system was implicated in the etiology of hypertension in these rats. In addition, an increase in DA-D1R and D2R was observed in young SHR and an increase of DA-D1R in the nucleus accumbens in adult SHR (Kirouac and Ganguly, 1993). The mRNA for the D2R were also increased in the nucleus accumbens and olfactory tubercles in young SHR, two major afferents to the VTA (Vaughan *et al.*, 1999). Autoradiography studies in young DOCA-salt rats have demonstrated an increase of D2R in the striatum and olfactory tubercles (Le Fur *et al.*, 1981). It seems therefore that the increased expression of DA receptors is not specific to SHR.

In normotensive rats, the activation of the baroreflex by electrical stimulation of aortic depressor nerve and by intravenous injection of phenylephrine altered the firing rate of DA neurons in the VTA (Kirouac and Ciriello, 1997). Neurons in the VTA were also sensitive to changes in blood pressure. Furthermore, studies reported that electrical stimulation of the VTA in anesthetized rats, as well as chemical stimulation with a stable analog of SP DiMe-C7 in conscious rats, increased blood pressure and this effect was blocked by the i.v. injection of DA-D1R, DA-D2R antagonists and vasopressin V1 antagonist (Cornish and van den Buuse, 1994, 1995). Furthermore, the DA agonist quinpirole injected intravenous (i.v.) induced a significant centrally-mediated increase in blood pressure in conscious rats (van den Buuse *et al.*, 1996) and electrical stimulation of central DA pathways also increased blood pressure (Cornish and van den Buuse,

1994). In addition, studies with chemical stimulation of the VTA enhanced cellular activity, which caused an increase in the expression of c-fos in the mesolimbic and mesocortical DA system (Swanson, 1982). Another study also with chemical stimulation of the VTA showed the c-fos expression, which was markedly increased in the supraoptic nucleus, but not paraventricular nucleus of the hypothalamus which are two regions that play a major role in vasopressin production.

5. The kallikrein-kinin system

5.1 Brief history

About 80 years ago, the researchers Emil Karl Frey, Heinrich Kraut and Eugene Werle, from the Max Planck Institute in Munich tried to identify the enzyme responsible for the release of kinins. While attempting to identify a substance that may be responsible for the anuria in patients, Frey (1926) observed that intravenous injection of human urine in dogs caused a drop in blood pressure (BP). French physiologists Abelous and Bardia (1909) had previously observed this phenomenon. Kraut *et al.* (1930) isolated that substance, which was found in several organs and mainly in the pancreas. This substance was named kallikrein (from the Greek kallikreas meaning pancreas), which on a preparation of the guinea-pig ileum, produced a smooth muscle contraction only in the presence of serum (Werle *et al.*, 1937). The kallikrein was in German called "Darmkontrahierender Stoff" (substance that contracts the intestine) or DK

substance; the DK is now named kallidin. In 1948, led by Maurício Rocha e Silva, three Brazilian physiologists and pharmacologists working at the Instituto Biológico in São Paulo, Brazil discovered bradykinin (BK) in attempting to determine the mechanisms involved in the septic shock produced by venom extracted from *Bothrops jararaca* (Brazilian lancehead snake) (Rocha e Silva *et al.*, 1949). As the extract contained kallikrein, the venom alone had no effect on the smooth muscle of the guinea pig ileum, but it produced its contraction in the presence of blood. In comparison to histamine and acetylcholine, the snake venom induced in intestinal smooth muscle a slow contraction. Rocha e Silva *et al.* named this substance “bradykinin”, a name from the suffix kinin (for movement) and the prefix brady (for slow) in Greek. Like kallidin (KD), BK also caused hypotension when injected in dogs. Within a short period of time, BK was isolated and purified. This process confirmed that it was a peptide (Andrade and Rocha e Silva, 1956, Elliot *et al.*, 1960). At the same time, the structure of KD was identified by the team of Werle (1961), and it was realized that KD was an extension of BK (Lys-BK). The similarity of their structure and their biological effects demonstrated that they belong to the same family. Thus, the two major kinins were found and their precursors were identified as the high molecular weight kininogen (HMWK) and low molecular weight kininogen (LMWK) by Habermann (1963). There is also T-kinin, which is found only in rats (Okamoto and Greenbaum, 1983 a, b). All these peptides were produced during tissue injury or during an infection, and they acted locally at the site of inflammation. The development of selective agonists

and antagonists has suggested the existence of two types of kinin receptors, called B1 and B2 (Regoli and Barabé, 1980). This classification was subsequently confirmed by cDNA cloning of B2R in rat by McEachern *et al.* (1991) and B1R in human by Hess *et al.* (1994).

In 1960, Rocha e Silva *et al.* identified BK as a potent vasodilator and observed that BK could increase BP at high doses. This led researchers to propose a central action for BK. Shortly after, it has been demonstrated in a model of cross circulation in dogs that kinins may act on the cardiovascular system by a central mechanism (Benetato *et al.*, 1964). Several studies in molecular biology and neuroanatomy followed, and helped identification and isolation of all components of the kallikrein-kinin system in most parts of the CNS. However, the physiological and pathological role of these peptides in the CNS remains largely unknown.

BK produces the four cardinal signs of inflammation: vasodilatation, increased vascular permeability, heat and pain. Moreover, kinins directly interact with the immune system, by stimulating the sensory C-fibers, along with the release of potent proinflammatory mediators, including prostanooids, tachykinins, cytokines, free radicals and nitric oxide (NO) (Calixto *et al.*, 2000; Couture *et al.*, 2001). Extensive research was undertaken, which revealed a role for kinins in inflammation. On the other hand, kininase II, the main catabolic enzyme of kinins was found to be identical to ACE (Erdos and Yang, 1970). This discovery opened an important avenue of research for the pharmaceutical industry in the field of hypertension with the development of ACE inhibitors. Lately, kinins

have mainly been known for their involvement in vascular and inflammatory processes.

5.2 Kininogens

In humans, there are two types of kininogens synthesized mainly by the liver cells. The HMWK is a glycoprotein (α -globulin) circulating in plasma at concentrations of 70 to 90g/ml with a molecular weight of 88-120 kDa (Adam *et al.*, 1985). LMWK, a precursor of KD is a glycoprotein (β -globulin) circulating in plasma at concentrations of 170-220g/ml with a molecular weight of 50-68 kDa (Dulinski *et al.*, 2003). They are permanently present in the plasma or bound to the endothelial cells membranes and in the immune system such as platelets and neutrophils (Bhoola *et al.*, 1992).

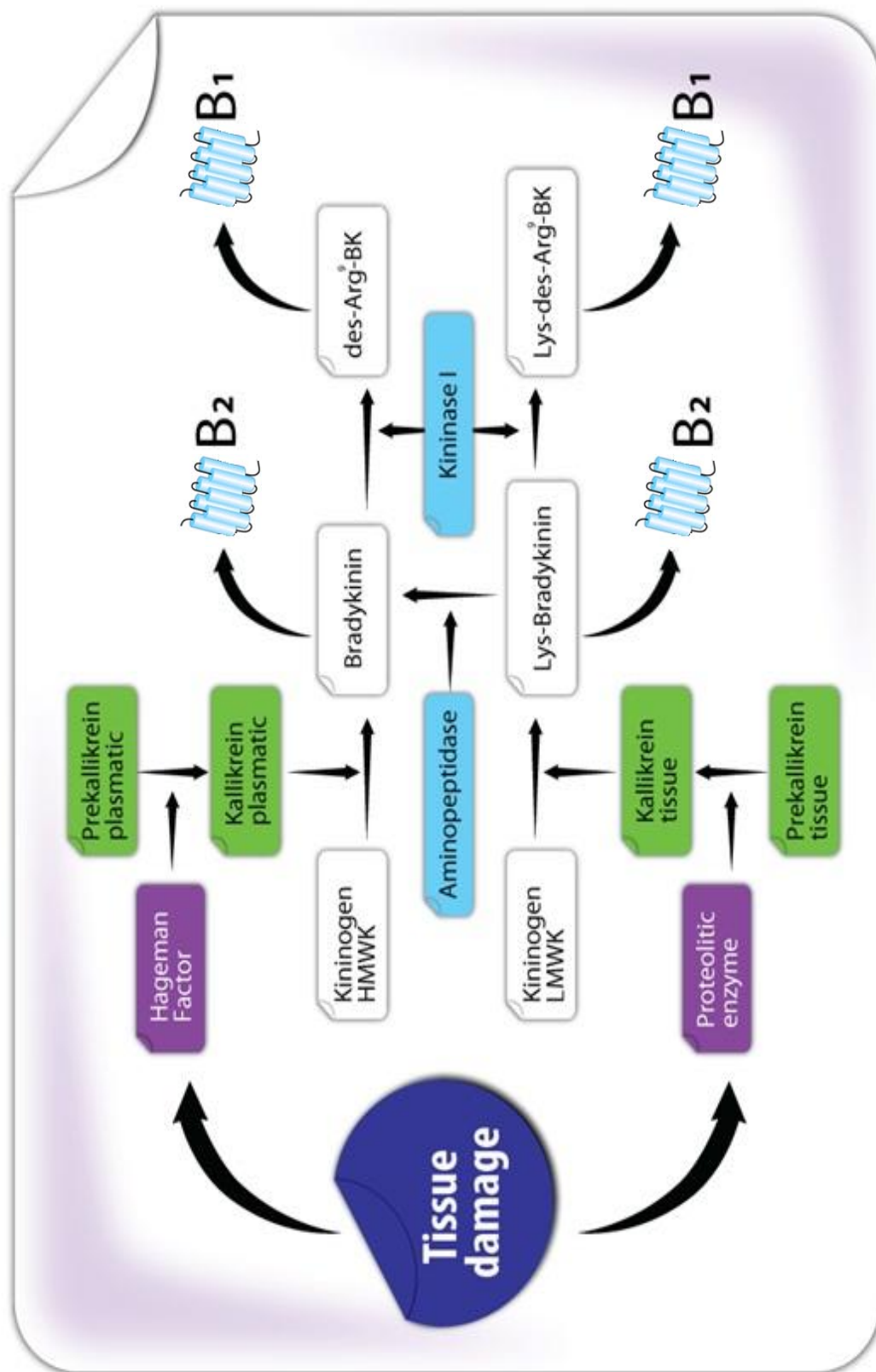
The third type is called T-kininogen (68 kDa) and is present only in rats. Two isoforms exist: TI and TII, with 96% homology between them (Okamoto and Grennbaum, 1983). The T-kininogen is distributed in different regions of the CNS, including the brainstem, the cortex, the mesencephalon and cerebellum (Marks *et al.*, 1988); the concentration is the highest in newborn rat and decreases gradually with growth (Damas *et al.*, 1992).

LMWK and HMWK are derived from alternative splicing of a single gene called gene K located on chromosome 3q26, while the T gene is responsible for the formation of T-kininogen. Genomic analysis of these two genes shows a similarity of approximately 90%, suggesting that they originate from a common ancestral gene (Nakanishi, 1987; Cole and Schreiber, 1992).

5.3 Formation of kinins by kallikreins

Kinins are generated by serine proteases named kallikreins. There are two major biochemical cascades leading to the release of kinins, in tissues and in blood. The tissue, cascade is initiated when kallikreins (25 to 45 kD) (Bhoola *et al.*, 1992) cleave the bond Met³⁷⁹-Lys³⁸⁰ and Arg³⁸⁹-Ser³⁹⁰ of LMWK to liberate kallidin (Fogaça *et al.*, 2004). In rats, there are 13 different tissue kallikreins (Gerald *et al.* 1986; Wines *et al.*, 1989, 1991). In blood, the cascade is initiated when the factor XII of coagulation (Hageman factor) becomes activated after contact with negatively charged surfaces such as damaged membranes or endotoxin. It interacts with the pre-complex which is pre-kallikrein and HMWK in the plasma. This interaction catalyzes the conversion of the pre-kallikrein into kallikrein and releases BK from HMWK (Figure 8). This kallikrein-kinin system participates actively in plasma coagulation and fibrinolysis protecting the endothelium (Linz *et al.*, 1995; Kaplan *et al.*, 1997). It is also involved in maintaining the vascular tone and the inflammatory process (Bhoola *et al.*, 1992). The tissue kallikrein is synthesized in its inactive form, pro-kallikrein, and transformed by several types of proteases. Unlike the plasma kallikrein, which is secreted by the liver, tissue kallikrein is synthesized in several tissues including pancreas, kidney, sweat glands, sub-maxillary glands and the epithelium of the cerebral ventricles ependyma (Bhoola *et al.*, 1992).

Figure 8. The kallikrein-kinin system.



Adapted from Rodi et al., 2005.

5.4 Kallikreins distribution

The presence of kallikrein in the brain was determined by radioimmunological techniques and *in situ* hybridization approaches (Chao and Chao, 1987). Kallikrein seems to be distributed in several regions of the rat CNS including cortex, brainstem and cerebellum. However, the highest concentration was found in the hypothalamus, pituitary and pineal gland. By immunohistochemistry, Kallikrein was observed in different regions of the brainstem including the oculomotor, red and reticular nuclei (Kizuki *et al.*, 1994). Immunocytochemically studies showed the presence of kallikrein in neuroglia, in neuron cell bodies of the supraoptic and paraventricular nucleus and of the ventromedial and posterior hypothalamus (Simson *et al.*, 1985; Snyman *et al.*, 1994). Furthermore, in prenatal and adult rats, kallikrein was found mainly inneuronal nuclei at the developing stage (Iwadate *et al.*, 2000). Kallikrein activity was also detected in human cerebrospinal fluid (CSF) (Scicli *et al.*, 1984). The administration of melittin (a compound, found in the bee venom, which activates membrane kallikrein) into the cerebral lateral ventricles (Nishimura *et al.*, 1980) increases the rate of immunoreactive kinins in the CSF. This is associated with an increase of BP in dogs (Thomas *et al.*, 1984). The treatment with melittin i.t. increases the density of BK-LI in the rat spinal cord, supporting the presence of kallikrein (Lopes and Couture, 1997).

5.5 Kininases

A variety of kininases were identified in the brain, such as aminopeptidases and enkephalinase (Camargo *et al.*, 1972; Schwartz *et al.*, 1981) and two additional endopeptidases: kininase A (metalloendopeptidase) and B (peptidyl dipeptidase) (Camargo *et al.*, 1973). In 1989, Orawski and Simmons showed that the degradation of BK in the brain synaptic membranes was due to the activity of three enzymes: 1) a form of the metalloendopeptidase which hydrolyses Phe⁵-Ser⁶, 2) a carboxypeptidase B which cuts the fragment Phe⁸-Arg⁹, and 3) peptidyl dipeptidase, which cleaves the fragment Pro⁷-Phe⁸, suggesting that BK has access to the synaptic membranes in the brain.

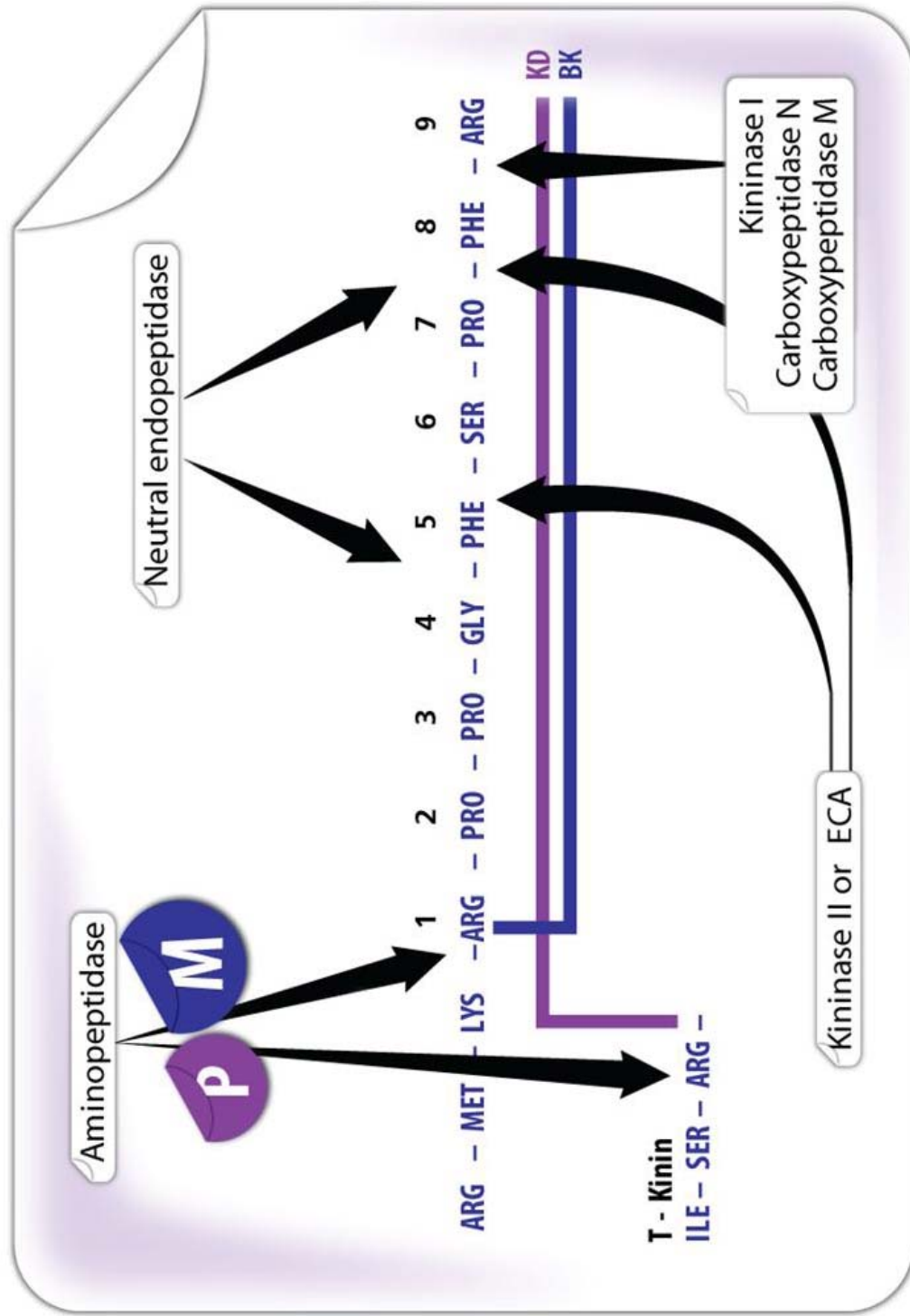
Kininase I is also known as carboxypeptidase N (CPN in plasma) and carboxypeptidase M (CPM on the plasma membrane of vascular cells). By removing the arginine in the C-terminal end of BK and KD, these enzymes yield des-Arg⁹-BK and des-Arg¹⁰-KD, which are biologically active peptides acting preferentially through the activation of the B1R.

Studies on the kininase distribution showed a very strong activity in the cerebellum and the striatum in comparison to other rat brain regions. For example, activity of the cerebellum and the striatum was about two times higher than in the cerebral cortex. The level of activity in regions of the mesencephalon, hippocampus, medulla oblongata and hypothalamus were not significantly different, and the spinal cord had less activity (Roth *et al.*, 1969; Cushman and Cheung, 1971; Yang and Neff, 1972; Kariya *et al.*, 1981; Elrod *et al.*, 1986).

Kininase II, better known as the angiotensin converting enzyme-1 (ACE) is a metalloprotease zinc glycoprotein which degrades BK into fragments BK(1-7), and BK(1-5) (Erdos, 1990) (Figure 9). ACE is found mainly at the surface of endothelial cells and in body fluids, such as semen, blood, urine, lymph and CSF (Bhoola *et al.*, 1992). In humans, two types of kininase II are found: 1) a protein of 150-180 kDa, present in most tissues, which is responsible for the degradation of two-thirds of the serum BK (Decarie *et al.*, 1996); and 2) a protein of 100-110 kDa which contributes to male fertility (Williams *et al.*, 1992; Fuchs *et al.*, 2005).

There are also aminopeptidases acting at the N-terminal ends of T-kinin and KD, and capable of transforming them into BK but these enzymes have no known role in the inactivation of kinins. The NEP is also found in the brain where it is known as enkephalinase (Erdos and Skidgel, 1989; Skidgel, 1992) (Figure 9).

Figure 9. Sites of proteolytic enzymes of kinins.



Adapted from Regoli and Barabe, 1980.

6. Kinin receptors

Analysis of kinin receptors has been progressing slowly, since in most biological preparations, kinins produce their actions indirectly via the release of factors derived from the endothelium or other cells. The rabbit aorta was the only preparation of smooth muscle to show a direct effect of BK (Garrett and Brown., 1972). However, BK caused an effect in 40% of the aortas, and the sensitivity was low in comparison to other biological preparations. The sensitivity to BK was strongly increased by the elimination of arginine in its C-terminal end (des-Arg⁹-BK) (Regoli *et al.*, 1977). This octapeptide was almost inactive on preparations such as guinea pig ileum, rat uterus, and spleen tissue frequently used to evaluate the activity of BK. The first BK antagonist ([Leu⁸]des-Arg⁹-BK) blocked the response of des-Arg⁹-BK and BK in the rabbit aorta but had no effect in other preparations. The existence of two types of receptors for BK was then proposed, based on the order of potency of agonists and the apparent affinity of competitive antagonists (Regoli *et al.*, 1977). That study provided the first pharmacological tools to characterize the two types of receptors and a large number of B1R and B2R agonists and antagonists were synthesized. The primary structure of BK is in Figure 9.

6.1 B1 receptors

Receptor induction is one of the cellular mechanisms displayed in response to stress. This process has been widely described for tyrosine kinase receptors, but has only rarely been described for G protein-coupled receptors (GPCR). The B1R is one of the members of GPCR, which can be

induced by a specific stimulus (Donaldson *et al.*, 1997). An increase of the temporal response to BK was described for the first time in a preparation of the dog saphenous vein in which BK produced vasoconstriction (Goldberg *et al.*, 1976).

Later, Regoli *et al.* (1977) first suggested the existence of B1R and B2R; they noticed that the sensitivity to des-Arg⁹-BK but not BK increased with the incubation time in the rabbit aorta. The same phenomenon was later observed in other vascular and non-vascular preparations (Regoli *et al.*, 1978; Marceau *et al.*, 1980; Couture *et al.*, 1981). Moreover, this phenomenon occurred in most *in vitro* preparations obtained from rabbit, rat or pig (Marceau, 1995) and also in human colon and ileum (Couture *et al.*, 1981; Zuzack *et al.*, 1996). Specifically, the tissues are insensitive to B1R agonist in the early hours of incubation, but gradually become more sensitive after a few hours of isolation *in vitro*. This phenomenon can be blocked by actinomycin D (an inhibitor of RNA synthesis), by cycloheximide (an inhibitor of protein synthesis), by tunicamycin (an inhibitor of N-glycosylation), by brefeldin A (an inhibitor of protein translocation), and glucocorticoids such as dexamethasone (Regoli *et al.*, 1978; Marceau *et al.*, 1980; Whalley *et al.*, 1983; Deblois *et al.*, 1988; Audet *et al.*, 1994; Campos and Calixto, 1994; Marceau, 1995; Haddad *et al.*, 2000).

The induction of the B1R involves increased expression of mRNA, protein translocation of the endoplasmic reticulum to the Golgi complex, an increased of transcription rate, a slower degradation or increased stability

of the mRNA, or both (Marceau, 1995; Haddad *et al.*, 2000; Sardi *et al.*, 2000).

The induction of B1R *in vivo* was first demonstrated in a model of cystitis in rats and subsequently on BP in rabbits treated with a sublethal dose of lipopolysaccharide (LPS) (Marceau *et al.*, 1984). The stimulation of B1R caused paw edema in the rat, after systemic treatment with LPS or treatment with a weakened strain of bacteria (*Mycobacterium bovis* bacillus Calmette-Guerin), local injection of proinflammatory cytokines or after surgical removal of the adrenal gland (Campos *et al.*, 1995; Campos *et al.*, 1997; Campos *et al.*, 1998; Cabrini *et al.*, 2001). The induction of B1R by heat stress in the rat aorta was also reported (Lagneux and Ribout, 1997; Lagneux *et al.*, 1998). Finally, the involvement of the B1R in the hypotensive effect of des-Arg⁹-BK or des-Arg¹⁰-KD after systemic treatment with LPS was observed in pig and monkey (Siebeck *et al.*, 1998; deBlois and Horlick, 2001).

The B1R plays an important role in chronic pain. The induction of B1R *in vivo* has been reported in inflammation and hyperalgesia models, after UV irradiation and intra-articular injection of Freund's adjuvant and cytokines in rats (Perkins *et al.*, 1993,1995; Davis and Perkins, 1994; Khasar *et al.*, 1995). The MAP kinase p38 is involved in the induction of functional B1R in a model of inflammatory hyperalgesia produced by intraplantar injection of IL-1 in rats (Ganju *et al.*, 2001). B1R KO mice showed a decreased in thermal hyperalgesia and mechanical allodynia induced by injection of Freund's adjuvant (Ferreira *et al.*, 2001). The non-peptide B1R

antagonist SSR240612 is anti-nociceptive in models of inflammatory and neuropathic pain induced by sciatic nerve ligation, UV irradiation, and injection of capsaicin (Gougat *et al.*, 2004). Autoradiography studies showed increases of B1R binding sites in the spinal cord and increased mRNA (*in situ* hybridization) in the thoracic spinal cord in streptozotocin (STZ) diabetic rats (Ongali *et al.*, 2004). B1R is likewise overexpressed in several brain areas, such as the hippocampus, amygdala, and throughout the cortex (Campos *et al.*, 2005). Selective B1R antagonists (R-715 and R-954) blocked thermal hyperalgesia in animal models of type 1 diabetes (STZ rats and BioBreeding/ diabetic-prone rats) (Gabra *et al.*, 2003, 2005a). Furthermore, the level of B1R mRNA was increased in endothelial cells incubated with high concentrations of glucose (Rodriguez *et al.*, 2006). A study by autoradiography in chronic glucose-fed rats (models of type 2 diabetes and insulin resistance) showed an increase in B1R binding in the spinal cord (El Midaoui *et al.*, 2005) and peripheral tissues (Lungu *et al.*, 2007). In glucose-fed rats, SSR240612 improved allodynia through a direct inhibition of B1R (Dias *et al.*, 2007).

6.1.2 Mechanism of the B1R induction

Various studies have demonstrated that the induction of B1R is controlled by the generation of some specific cytokines produced during tissue injury or stress (Marceau and Bachvarov, 1998a).

B1R is induced and overexpressed in the presence of cytokinins TNF- α , IL-1 β , IL-6, and IL-4 (Marceau *et al.*, 1998; deBlois and Horlick, 2001; Sardi *et*

al., 2002). The synthesis of IL-1 β is induced by inflammatory mediators that are stimulated by bacterial endotoxins or viral agents (Stylianou and Saklatvala, 1998).

Receptor-adaptor interactions lead to the recruitment and activation of kinases of the IL-1R-associated kinase (IRAK) family, which regulate positively or negatively, a complex signaling cascade leading to the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein kinases (MAPK), and to the production of defense molecules such as inflammatory cytokines (Meylan and Tschöpe, 2008). In addition, the intracellular signal triggered by IL-1 β stimulation can lead to several proteins of the family of MAP kinases such as p38, p42, p44, NIK, and SAPK JNK (Saklatvala *et al.*, 1999).

Subsequently, these proteins can activate a group of kinases called IKK1 and IKK2, which are responsible for controlling the expression of genes regulated by the NF- κ B. In addition, the response via IL-1 β may involve the production of cAMP and activation of CREB or the activation of the transcription factor (AP-1) via PKC stimulation (Barnes, 1998; Meylan and Tschöpe, 2008). All these pathways lead to inducible gene coding for the B1R. Binding sites for these transcription factors (AP-1, CREB and NF- κ B) are in the promoter region of the gene coding for B1R (Ni *et al.*, 1998b; Yang *et al.*, 1998). It was shown that mutations in the binding site for NF- κ B prevented the induction of B1R by LPS, IL-1 β and TNF- α in human vascular smooth muscle cells (Ni *et al.*, 1998a). The involvement of NF- κ B in the induction of functional B1R was shown in the rabbit aorta after

treatment with LPS (Medeiros *et al.*, 2001). A treatment with pyrrolidine dithiocarbamate (PDTC, inhibitors of NF- κ B) inhibited the contractile response to des-Arg⁹-BK in the rabbit aorta. The same observations were reported in the rabbit aorta incubated with IL-1 β (Sabourin *et al.*, 2002). These authors showed that the inhibition of NF- κ B reduces the expression of mRNA encoding the B1R using fresh or cultured rabbit aortic smooth muscle cells.

Several other cytokines, such as TNF α , IL-2 and IL-8, and some growth factors, are also capable of inducing the upregulation of B1R (Marceau *et al.*, 1998b; Campos *et al.*, 1999). However, this effect is likely to be secondary to the production of IL-1 β (Marceau *et al.*, 1998b). Some studies suggest that the B1R could also be induced by its own agonist in human lung fibroblasts (IMR 90) (Schanstra *et al.*, 1998; Phagoo *et al.*, 1999).

6.1.3 B1R agonists

Des-Arg¹⁰-KD and des-Arg⁹-BK, the two natural agonists for the B1R, were studied in several systems: contractile (rabbit jugular vein and rat portal vein) and relaxing (dog renal artery devoid of endothelium). All results obtained with these studies reveal that the activity of des-Arg¹⁰-KD is higher than that of des-Arg⁹-BK in human, rabbit and pig (Regoli *et al.*, 2001). This may be explained by a co-evolution of the respective genes encoding B1R and kininogens to keep optimal physiological function following the activation of B1R (Marceau *et al.*, 1998b; Regoli *et al.*, 2001). This theory is supported by the fact that the sequence of BK in rat and

mouse kininogen is preceded by an arginine, while the human kininogen contains the sequence of Lys-BK (Furuto-Kato and al., 1985, Hess *et al.*, 1996). The synthetic B1R agonist, Sar[DPhe⁸][des-Arg⁹]BK, has high affinity and selectivity for B1R (Drapeau *et al.*, 1991) and it is more potent than des-Arg⁹-BK, but less than Lys-des-Arg⁹-BK (Levesque *et al.*, 1995). Moreover, the presence of B1R in human brain endothelial cells (HBECs) was upregulated on the surface of the HBECs by molecules released during inflammatory response. The signaling via this receptor can regulate blood-brain barrier (BBB) permeability (Prat *et al.*, 2000) and to show its involvement in the cardiovascular effect produced by i.c.v. injection of kinins in SHR and WKY (Emanuelli *et al.*, 1999). These agonists are therefore a good instrument to demonstrate the presence of functional B1R.

6.1.4 B1R antagonists

The first B1R antagonist, [Leu⁸]-des-Arg⁹-BK, was developed by Regoli's team (1977). Lys-[Leu⁸]-des-Arg⁹-BK and [Leu⁸]-des-Arg⁹-BK have been the two most used B1R antagonists to study the contribution of B1R in several experimental models and diseases such as hypertension, type 1 diabetes, sepsis and inflammation (Marceau *et al.*, 1998b). Due to their rapid metabolism these antagonists have some limitations, since they maintain a residual agonist activity in some species. Regoli's team also developed the B1R antagonist R-715 (Gobeil *et al.*, 1996b). This antagonist showed no partial agonism and is a competitive antagonist in human, rabbit and mouse (Regoli *et al.*, 1998). R-715 has enabled to suggest the

involvement of B1R in the CNS of SHR (Emanuelli *et al.*, 1999) and in two rat models of epilepsy (Bregola *et al.*, 1999) (Table VII).

LF220542, a non-peptide B1R antagonist, showed high affinity in human and mouse. Moreover, the blockade of the B1R with LF220542 caused significant anti-nociceptive and anti-hyperalgesic actions in acute, visceral, inflammatory and experimental neuropathic pain. This data supports the effects seen by Porreca *et al.* in B1R KO mice, showing the selectivity of LF220542 for B1R and the potential development of antagonists for pain treatment (Porreca *et al.*, 2006) (Table VII).

The non-peptide B1R antagonist SSR240612 was developed by Sanofi-Aventis and is active orally (Gougat *et al.*, 2004). The intraperitoneal administration of SSR240612 in a dose-dependent way reduced the paw edema caused by des-Arg⁹-BK in mice treated with IL-1 β . Moreover, oral administration of SSR240612 decreased paw edema induced by des-Arg⁹-BK in mice pretreated with IL-1 β and ear edema caused by capsaicin application. In 2006, Costa *et al.* showed that SSR240612 given by gavage reduced the total cell and neutrophil counts in the pleurisy model induced by carrageenan in mice. This was the first non-peptide B1R antagonist with confirmed *in vivo* efficacy when administrated by gavage. In addition, SSR240612 prevented intestinal tissue damage and neutrophil accumulation after splanchnic artery occlusion-reperfusion injury. This result suggests that SSR240612 has relevant anti-inflammatory actions (Campos *et al.*, 2006).

Dias *et al.* (2007) showed that B1R is implicated in tactile and cold allodynia in glucose-fed rats, an experimental model of insulin resistance. SSR240612 administrated by gavage reversed in a dose-dependent way tactile and cold allodynia. Hence, non-peptide B1R antagonists such as SSR240612 with oral bioavailability may represent new therapeutic tools for the treatment of neuropathic pain.

Table VII. Chemical formulae of B1R antagonists

Antagonists	Sequences	References
Leu ⁸ -des-Arg ⁹ -BK	Leu ⁸]des-Arg ⁹ -BK	Regoli and Barabe, 1980
[des-Arg ¹⁰]-Hoe 140	Des-Arg ⁹ -D-Arg-[Hyp ³ ,Thi ⁵ , D-Tic ⁷ ,Oic ⁸]-BK	Wirth <i>et al.</i> , 1991
R-914	AcLys-Lys-[α (Me)Phe ⁵ , D- β Nal ⁷ ,Ile ⁸]des-Arg ⁹ -BK	Gobeil <i>et al.</i> , 1999a
R-715	AcLys-[D- β Nal ⁷ ,Ile ⁸]des-Arg ⁹ -BK	Gobeil <i>et al.</i> , 1996b
R-954	Ac-Orn-[Oic ² , α (Me)Phe ⁵ , D- β Nal ⁷ ,Ile ⁸]des-Arg ⁹ -BK	Gabra and Siriois, 2005b
LF-22-0542	N-[[4-(4,5-dihydro-1H-imidazol-2-yl)phenyl]methyl]-2-[2-[[4-methoxy-2,6-dimethylphenyl)sulfonyl]methoxy]ethoxy]-N-methylacetamidefumarate	Porreca <i>et al.</i> , 2006
SSR240612	[(2R)-2-[[[(3R)-3-(1,3-benzodioxol-5-yl)-3-[[6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl)amino]-3-(4-[[2R,6S)-2,6-dimethylpiperidiny]methyl]phenyl)-N-isopropyl-N-methylpropanamide hydrochloride]	Gougat <i>et al.</i> , 2004

6.2 B2 receptors

The B2R are expressed in many tissues such as: endothelial cells, fibroblasts, neurons, and smooth muscle (Bhoola *et al.*, 1992). This receptor is also responsible for: neurogenic inflammation, vasodilation, and increased vascular permeability. Furthermore, the activation of the B2R indirectly leads to an increase of cAMP / cGMP via the release of prostacyclin and NO, which causes vasodilation (Gabra *et al.*, 2003). BK causes neurogenic inflammation by activating sensory C-fibers, which release SP and calcitonin gene related peptide (CGRP). This also leads to the release of prostaglandins, mediators of mast cells (histamine and serotonin), and cytokines (Dray, 1997, Couture *et al.*, 2001).

6.2.1 B2R agonists

BK remains the most used agonist despite the fact that there are some synthetic agonists for the B2R. [Tyr(Me)⁸]-BK and [Hyp³, Tyr(Me)⁸]-BK are B2R agonists generally more active than BK in preparations containing B2R (Regoli *et al.*, 1977; Gaudreau *et al.*, 1981a; Gaudreau *et al.*, 1981b; Rhaleb *et al.*, 1991; Regoli *et al.*, 1991). Despite their greater affinity, these peptides have been little used because they are not commercially available. In addition, the fact that kinins produce four cardinal signs of inflammation: rubor (redness); calor (heat), tumor (swelling) and dolor (pain), the lack of interest in producing stronger B2R agonists were reported. However, the action of BK on the permeability of the BBB has raised clinical interest.

Indeed, BK increases the permeability of the BBB and might allow certain compounds such as anticancer agents to better penetrate the brain. For example, labradimil (Cereport®, formally known as RMP-7) has been developed as a B2R agonist with greater resistance to metabolism than BK (Straub *et al.*, 1994; Emerich *et al.*, 2001). This agonist was effective in increasing the chemotherapeutic agents' concentration inside brain tumors (Emerich *et al.*, 2001). The nonpeptide B2R agonist, FR190997, behaves as a full agonist on human, as partial agonist on rabbit, and as pure antagonist on pig B2R (Gobeil *et al.*, 1999b; Regoli *et al.*, 2001).

6.2.2 B2R antagonists

There are three generations of B2R antagonists. The first was initiated by Vavrek and Stewart, 1985, who made the following changes in the structure of BK: they replaced the proline in position 7 by one D-aromatic acid, D-phenylalanine (D-Phe) that produced [D-Phe⁷]-BK. Although this antagonist has low affinity, the substitution of phenylalanines 5 and 8 by β -(2-thienyl)alanine(Thi) forming the [Thi^{5,8},D-Phe⁷]-BK or the substitution of a trans-4-hydroxyproline (Hyp) at position 2 or 3 forming [Hyp³, Thi^{5,8}, D-Phe⁷]-BK improved the affinity (Rhaleb *et al.*, 1991).

Furthermore, the basic residues D-arginine or lysine at the N-terminal, increase the metabolism resistance. However, these compounds were quickly found to be unsuitable for the pharmacological characterization of kinin receptors for the following reasons: they acted as partial agonists in some tissue preparations and they have relatively weak antagonist activity

(pA₂ of 5.9 to 6.2), and low selectivity for B2R. They also caused the release of histamine both *in vitro* and *in vivo* (particularly those with a Lys or Arg in the N-terminal) (Devillier *et al.*, 1988; Lawrence *et al.*, 1989; Rhaleb *et al.*, 1991; Regoli *et al.*, 1993). In 1991, Regoli *et al.* produced another series of analogues [D-Phe⁷]-BK by substituting a leucine in position 8. The most interesting compounds are D-Arg-[Hyp³, D-Phe⁷, Leu⁸]-BK and Ac-D-Arg-[Hyp³, D-Phe⁷, Leu⁸]-BK. They have a greater affinity for the B2R (including the rabbit B2R), little or no residual agonist activity and a relatively low pA₂ (pA₂ is the dose of antagonist that requires a 2-fold increase in agonist concentration) for the B1R.

The second generation of B2R antagonist is characterized by the development of Hoe 140 (D-Arg-[Hyp³, Thi⁵, D-Tic⁷, Oic⁸]-BK) by the company Hoechst and is also known under the brand name of Icatibant® (Hock *et al.*, 1991). This B2R antagonist has high affinity and selectivity for B2R. In fact, its pA₂ is the 8-fold or higher in most species studied (Regoli, 2000), including rat (Meini *et al.*, 2000). Furthermore, the Hoe 140 has a long duration (>60 minutes) due to its protection against metabolism and its long interaction with B2R. Moreover, the novelty with Hoe 140 was the addition of two artificial amino acids at positions 7 and 8 (DTic: 1,2,3,4 acid, tetrahydroisoquinoline-3-carboxylic acid; OIC acid [3aS, 7AS]-octahydroindole -2 - carboxylic acid), which improved affinity and resistance (Hock *et al.*, 1991; Gobeil *et al.*, 1996a). Hoe 140 is a B2R antagonist competitive just in human, not in rabbit and pig (Regoli *et al.*, 1998).

The structure of Hoe 140 enables its use as radioligand ($[^{125}\text{I}]$ HPP-Hoe 140 and $[^{125}\text{I}]$ para-iodophenyl Hoe 140 ($[^{125}\text{I}]$ PIP-Hoe 140). These ligands have been used successfully to reveal by autoradiography the presence of B2R in central and peripheral tissues of guinea pig, sheep and rat (Murone *et al.*, 1996, Murone *et al.*, 1997; Cloutier *et al.*, 2002; Ongali *et al.*, 2006), in the vagus nerve in rat and human (Krstew *et al.*, 1998), in the human brainstem, and in mouse cancer cells (Wu *et al.*, 2002).

Finally, the third generation of B2R antagonists are non-peptide molecules designed for oral administration in the treatment of chronic inflammatory diseases. The first prototype, WIN64338, synthesized by the pharmaceutical company Sterling Winthrop (Salvino *et al.*, 1993), had a promising future with its strong affinity for B2R in the guinea pig ileum. However, this component is not specific since it has a strong affinity for muscarinic receptors (Sawutz *et al.*, 1994). Moreover, in comparison to Hoe 140, which has a very high affinity in several species, WIN64338 has a variable affinity between species and is almost inactive in human (Regoli *et al.*, 1996, 1998).

Shortly after, FR173657 was proposed by Fujisawa Pharmaceutical Company (Inamura *et al.*, 1997). It represents the first orally active B2R antagonist in several species. FR173657 inhibits the hypotension and plasma protein extravasation induced by BK and the nociceptive behavioural response following intraplantar injection of BK in rats (Griesbacher and Legat, 1997; Griesbacher *et al.*, 1998). The vascular effects of kinins are also inhibited by this antagonist in experimental models of

pleurisy (Majima *et al.*, 1997), pancreatitis and cystitis (Griesbacher *et al.*, 2000) and allergy in guinea pig (Mori and Imamura, 1998). Therefore, FR173657 represents an excellent pharmacological tool. Based on its structure, the pharmaceutical company Fournier developed the non-peptide antagonist LF16-0687 which seems very promising from results obtained in various animal models of brain injury (Rachinsky *et al.*, 2001; Plesnila *et al.*, 2001) (Table VIII).

Table VIII. Chemical formulae of B2R antagonists

Antagonists	Sequences	References
HOE 140	D-Arg-[Hyp ³ ,Thi ⁵ ,D-Tic ⁷ ,Oic ⁸]-BK	Hock <i>et al.</i> , 1991
FR173657	(8-[3-[N-(E)-3-(6-acetamidopyridin-3-yl)acryloylglycyl]-N-m ethylamino 2,6-dichlorobenzoyloxy]-2-methylquinoline)	Aramori <i>et al.</i> , 1997a
LF16-0687	(1-[[2,4-dichloro-3-[[[(2,4-dimethylquinolin-8-yl)oxy] methyl]phenyl]sulfo N-[3-[[4-(aminoimethyl) phenyl] carbonylamino]propyl]-2(S)- pyrrolidinecarboxamide)	Pruneau <i>et al.</i> , 1999b

6.2.3 B2R expression

B2R is traditionally known to have a constitutive expression in a wide variety of tissues including endothelial cells, fibroblasts, neurons, epithelial cells, skeletal muscle and smooth muscles cells (Dendorfer *et al.*, 1999; Couture and Lindsey, 2000). The physiological responses induced by activation of B2R are subjected to tachyphylaxis due to the temporary desensitization of B2R, which involves phosphorylation followed by receptor internalization (Dengler *et al.*, 1990; Munoz *et al.*, 1992, 1993; Phagoo *et al.*, 1999; Faussner *et al.*, 1999; Marceau *et al.*, 2001). Moreover, it has been suggested that ACE inhibitors enhance B2R activation of endogenous kinins not only by preventing degradation, but by preventing also B2R internalization and desensitization (Munoz and Leeb-Lundberg, 1992; Minshall *et al.*, 1997a, b; Marcic *et al.*, 1999). In 2000, Marcic and Erdös suggested that ACE inhibitors do not act directly on B2R, but on a cross-talk mechanism between ACE and B2R.

B2R expression is increased in human synovial cells by IL-1 β (Barton *et al.*, 1992), in smooth muscle cells by cAMP (Dixon, 1994) or by PDGF (Platelet-derived Growth Factor) (Dixon *et al.*, 1996), and in the dorsal horn ganglia by NGF (Nerve Growth Factor) and GDNF (Glial-Derived Neurotrophic Factor), via TrkA (tyrosine kinase receptor A) (Lee *et al.*, 2002). B2R expression is also increased in smooth muscle cells of the human bronchi (Schmidlin *et al.*, 1998) and in vascular smooth muscle cells by high concentrations of glucose via PKC (Christopher *et al.*, 2002), in human

fibroblasts WI-38 by IL-10 (Phagoo *et al.*, 2000), in human embryonic lung fibroblasts (HEL 299) by TNF- α and IL-1 via p38 MAPK (Haddad *et al.*, 2000), myocardial ischemia (Tschöpe *et al.*, 2000).

6.3 Cloning of kinin receptors

Deoxyribonucleic acid (cDNA) cloning and genes encoding of the kinins receptors confirmed the pharmacological classification but did not validate the existence of B3, B4 and B5 types.

6.3.1 B1R cloning

B1R was cloned and its sequence was established in human (Menke *et al.*, 1994; Yang and Polgar, 1996, Chai *et al.*, 1996), in mouse (Pesquero *et al.*, 1996), in rat (Jones *et al.*, 1999), in rabbit (MacNeil *et al.*, 1995) and in dog (Hess *et al.*, 2001). However, the potency of agonists differs between species. While des-Arg⁹-BK has more affinity in rodents and in dogs, B1R in human and in rabbits have a preference for analogues with a group Lys in the N-terminal position (Lys-des-Arg⁹-BK). There are three potential sites of N-glycosylation and for phosphorylation by PKA and PKC. The gene encoding B1R in humans and in rats were analyzed, and it was found the TATA box sequence and a large number of binding sites for transcription factors related to inflammation such as AP-1, NF- κ B and CREB (Bachvarov *et al.* 1996; Ni *et al.*, 1998a, b; Jones *et al.*, 1999).

In all species studied, the B1R presented between 30% and 36% homology with the B2R receptor. The studies on the structure and genomic organization showed that the respective genes to these two receptors are

constituted of 3 exons separated by two introns and that they are localized very close to each other on the same human chromosome 14q32 (Kammerer *et al.*, 1995; Bachvarov *et al.*, 1996; Yang and Polgar, 1996).

6.3.2 B2R cloning

The cDNAs encoding the B2R were cloned in rat (McEachern *et al.*, 1991; Pesquero *et al.*, 1994, Wang *et al.*, 1994), in human (Hess *et al.*, 1994a,b; Park *et al.*, 1994, Kammerer *et al.*, 1995), in mouse (McIntyre *et al.*, 1993; Hess *et al.*, 1992, 1994; Ma *et al.*, 1994b), in guinea pig (Farmer *et al.*, 1998), in rabbit (Bachvarov *et al.*, 1995) and in dog (Hess *et al.*, 2001).

For all species studied, the sequences have a homology of 80% or more. The order of potency of agonists and antagonists was the same but with differences in affinities between species. The analysis showed sequences of potential N-glycosylation and phosphorylation sites by PKC and PKA in all of these species. The genomic analysis suggested a single copy of the gene coding for the B2R in humans (Ma *et al.*, 1994a). The consensus sequence TATA box has been identified as potential sites for binding the elements of response to cAMP (CREB) and IL-6 on the gene encoding the B2R in human, rat and mouse (Wang *et al.*, 1994, Ma *et al.*, 1994a, b, Pesquero *et al.*, 1996).

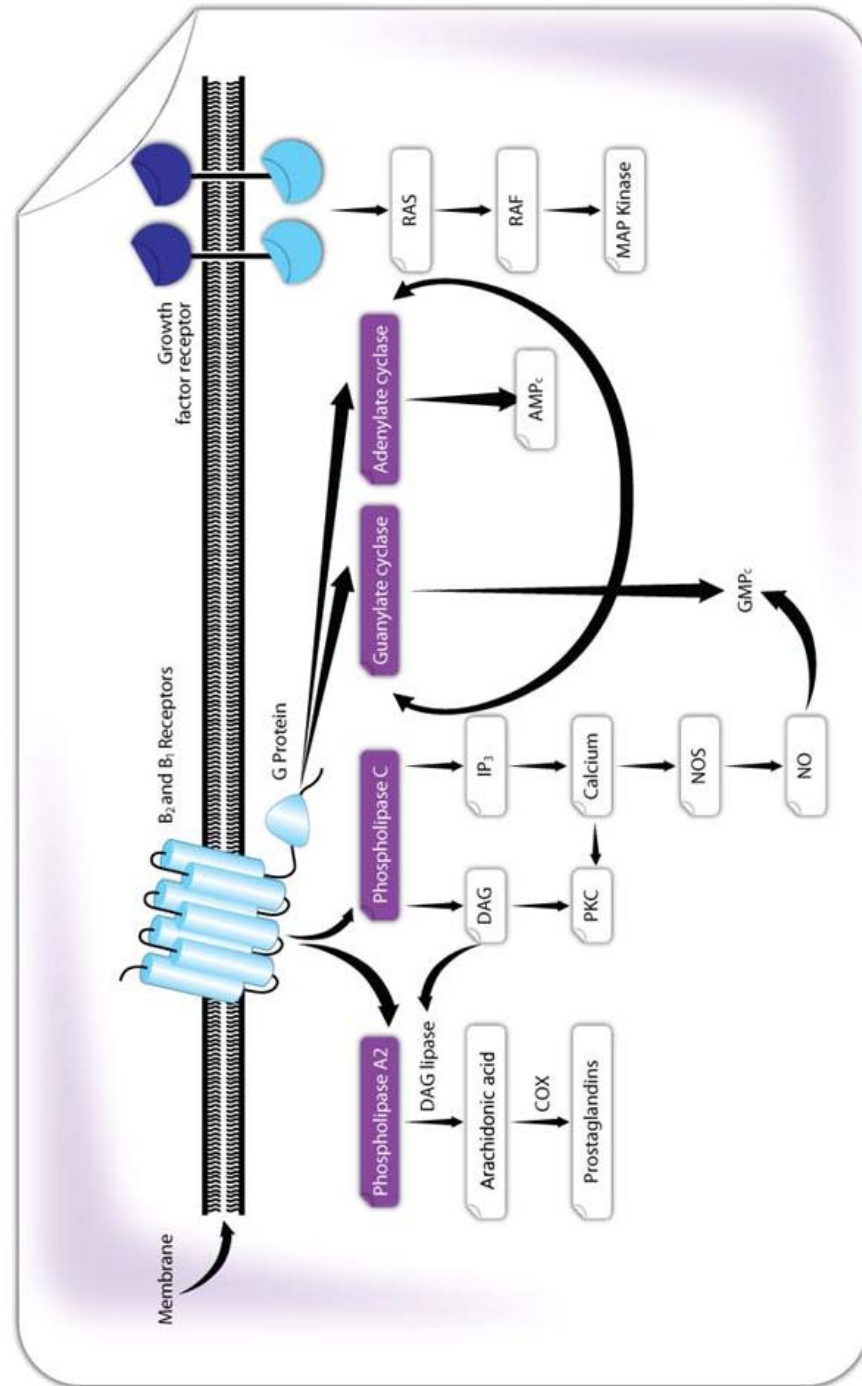
6.3.3 Intracellular signaling pathways

BK activates the "classical" signaling pathways which belong to the GPCR family by recruiting proteins G_{ai} and G_{aq} . The B2R also signals through G_{ay} (Jones *et al.*, 1995) and G_{ot} (Ewald *et al.*, 1989). Depending on the cell

type, BK stimulates a variety of intracellular signaling mechanisms. These include direct or indirect accumulations of cyclic AMP and cyclic GMP, and the activation of phospholipase A₂, C and D (Figure 10). B2R activation by BK promotes a rapid release of IP₃ and DAG, presumably by activating a specific PLC (Yano *et al.*, 1985). IP₃ and DAG release, in turn, leads to the liberation of Ca²⁺ from intracellular stores and the activation of PKC (Figure 10).

The activation of "alternative" new pathways apparently independent of G-protein was shown for the B2R (Bascands *et al.*, 2003). A direct interaction was showed between the B2R and enzymes such as phospholipase C γ , neuronal NO synthase (nNOS), endothelial NO synthase (eNOS) and tyrosine phosphatase SHP2 (AbdAlla *et al.*, 2005). The interaction of B2R with eNOS allows the recruitment of protein kinase Akt phosphorylation and thus controls eNOS and NO production. The interaction between the B2R and tyrosine phosphatase SHP2 is involved in the antiproliferative effect of BK (Duchene *et al.*, 2002), it involves a sequence ITIM (immunoreceptor tyrosine-based inhibitory pattern). The mutation of the Tyr residue of the ITIM deletes interaction and the inhibition of the proliferation by BK. Equally unusual for a GPCR is the formation of a heterodimer, bringing the B2R and the AT1 receptor of angiotensin II. The formation of this heterodimer makes the complex resistant to oxygen radicals and alters endocytosis. A consequence would be the AT1 receptor hypersensitivity for angiotensin II (Hansen *et al.*, 2009).

Figure 10. Signaling Mechanism of kinin B1R and B2R.



Adapted from Gabra et al., 2003.

A study using Chinese Hamster Ovary cells transfected with the human B1R gene showed that [³H]des-Arg¹⁰-LBK binding to the human B1R leads to the generation of inositol phosphate (IP) and transient increases in intracellular calcium, demonstrating that the receptor was coupled to PLC activation. This is consistent with studies also demonstrating B1R-mediated activation of PLC in cells from other species and confirms that B1R is coupled to this signaling pathway (Austin *et al.*, 1997) (Figure 10).

Several studies revealed that the B1R and B2R are linked to the activation of tyrosine kinases, phosphatases and the MAP kinase (mitogen-activated protein kinase) (Liebmann and Böhmer, 2000; Liebmann, 2001). For instance, the inhibitory effect of BK on cell proliferation which is mediated by B2R is associated with an increase in tyrosine phosphatase activity and a decrease in tyrosine phosphorylation (Alric *et al.*, 2000).

The activation of B1R and B2R may also cause subsequent activation of the phosphatidylinositol 3-kinase and sphingosine kinase (Liebmann and Böhmer, 2000; Liebmann, 2001; Blaukat and Dikic, 2001).

The difference between the two mechanisms of transduction of kinin receptors lies in their way of increasing intracellular calcium. B2R activation preferentially mobilizes the intracellular calcium, while the activation of the B1R seems to be associated with an influx of calcium (Bascands *et al.*, 1996). The temporal pattern of intracellular events in cells that express both types of receptors is also interesting. The increase in intracellular Ca²⁺ produced by stimulation of the B1R is more persistent

and less subject to desensitization in endothelial cells in rats (Mathis *et al.*, 1996). In the same way, activation of PLC is more persistent with the stimulation of the B1R (Austin *et al.*, 1997).

7. Cardiovascular effects of kinins

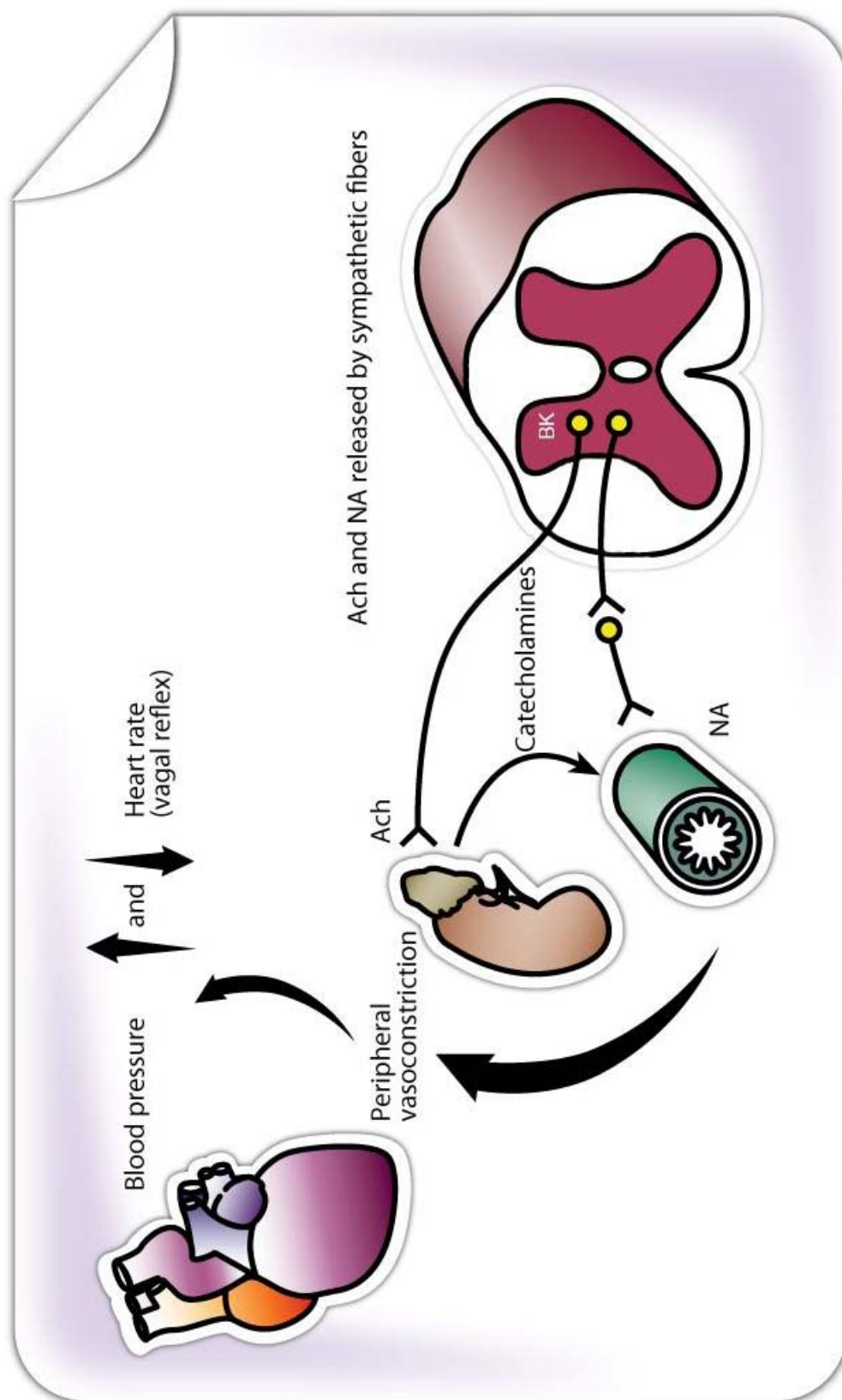
Kinins in the periphery are vasoactive peptides that cause vasodilation and a decrease in BP (Bhoola *et al.*, 1992). Many studies have identified several sites of action for kinins in the CNS having an effect on the cardiovascular system: the lateral area of the septum (Corrêa and Graeff, 1976), the hypothalamic nuclei (Diz and Jacobowitz, 1984; Diz, 1985), the ventral portion of the III ventricle (Lewis and Phillips, 1984), the periventricular areas of the dorsal medulla oblongata, the NTS, the paratrigeminal nucleus (Pa5), the area postrema, the dorsal motor nucleus of vagus, the trigeminal nucleus (SP5) (Lindsey *et al.*, 1988, 1997; de Sousa Buck *et al.*, 2002; Privitera *et al.*, 2003) and the spinal cord (Lopes and Couture, 1992; Cloutier *et al.*, 2002, Cloutier *et al.*, 2004). The injection of BK in these structures increased BP by increasing the activity of the sympathetic nervous system. Moreover, the i.t. injection of BK, KD and T-kinin to the ninth thoracic segment in unanesthetized rats increased BP and decreased HR via B2R (Lopes and Couture, 1992) (Figure 11). Antagonists selective for B2R showed a specific and reversible blockade of the pressor effects produced by administration of BK in the lateral septal area (Corrêa and Graeff, 1976), the IV ventricle

and the Pa5 (Lindsey *et al.*, 1989; Martins *et al.*, 1991) and the spinal cord (Lopes and Couture, 1992).

In 2004, Cloutier *et al.* showed the cardiovascular effects of BK injected into the i.c.v. in SHR and WKY. These effects confirmed previous studies suggesting that central administration of BK and related peptides causes cardiovascular changes through the activation of B2R in both, hypertensive and normotensive and rats (for review, see Couture and Lindsey, 2000). This effect also was blocked by the selective B2R antagonist Hoe-140. However, the central administration of the Hoe-140 did not caused changes on cardiovascular effects in SHR and WKY. Moreover, the pressor effects caused by BK were partly mediated by the activation of the sympathetic nervous system (Bunag and Takahashi, 1981; Takahashi and Bunag, 1981; Qadri *et al.*, 1999), and it is possible that BK-induced pressor responses originate at least partly from an exaggerated sympathetic tone in SHR. Changes in kininase II activity cannot provide an explanation for the hypersensitivity to BK since it was found higher in the CSF of adult SHR, suggesting a higher metabolic activity for kinins (Israel and Saavedra, 1987).

The involvement of B1R in hypertension is controversial. In SHR, i.c.v. injection of Sar[DPhe⁸][des-Arg⁹]BK, a B1R agonist, increased BP while the B1R antagonist, R-715, decreased BP (Emanuelli *et al.*, 1999). However, the same pharmacological approach did not confirm the involvement of B1R in the central control of BP in SHR (Cloutier *et al.*, 2004).

Figure 11. Diagram of the cardiovascular effects of BK on B2R in the spinal cord.



Adapted from Couture et al., 1995.

8. Kinin receptors in the CNS

Several techniques have been used to localize kinin receptors in the CNS including autoradiography on tissue sections, detection of mRNA by *in situ* hybridization and immunocytochemistry. The first evidence of the presence of B2R in the CNS was reported in 1981, by Innis *et al.* who observed saturably high affinity binding sites for [³H]-BK on brain membrane preparations from rat, guinea pig and bovine cerebellum.

Binding sites with high affinity for B2R were detected using [¹²⁵I]-Tyr⁸-BK in many systems: on nerve cells from brain of newborn rats (Lewis *et al.*, 1985), cultured astrocytes from the cortex, brainstem, spinal cord of fetal rats and cerebellum of newborn rats (Cholewinski *et al.*, 1991), homogenates of the bovine hippocampus (Kozlowski *et al.*, 1988) and guinea pig brain (Fujiwara *et al.*, 1988).

Kinin receptor binding sites were also observed using [³H]-BK on the endings of sensory C-fibers in the spinal gelatinous substance and the trigeminal nucleus (Steranka *et al.*, 1988). The ligand [³H]-BK showed a high density of binding in the medulla oblongata and spinal cord, a moderate density in the cerebral cortex, and a low density in the hippocampus as well as in other brain regions of guinea pigs (Fujiwara *et al.*, 1989). Sharif and Whiting (1991) confirmed the identification of B2R and its regional distribution in the CNS. With the ligand [¹²⁵I]-Tyr⁸-BK, Privitera *et al.* (1992) showed by autoradiography the presence of BK receptors in the region of the medulla oblongata, specifically the nucleus of the solitary tract (NTS), the area postrema, the dorsal motor nucleus of the

vagus nerve (X) and the spinal trigeminal nucleus. Their study confirmed the regional distribution observed by Fujiwara *et al.* (1989) for the medulla and the protuberance, but they failed to detect specific binding on BK in other brain regions (cerebellum and cerebral cortex).

The anatomical distribution and pharmacological characterization of B2R in the different laminae of the spinal cord were studied in rat and guinea pig. Although the number of B2R binding sites was higher in guinea pig in comparison to rat, the segmental and laminae distributions were similar in both species (Lopes *et al.*, 1993; 1995).

Treatment with capsaicin (a neurotoxin for sensory C-fibers) in the newborn rat caused a significant decrease in the density of binding sites for [¹²⁵I]-Tyr⁸-BK in laminae I and II of the spinal cord. This suggests a presynaptic location on primary sensory afferents and presumably on sensory neuron terminals of small diameter (C and A δ) in the superficial laminae of the dorsal horn (Lopes *et al.*, 1995). These authors also showed that treatment with intrathecal injection of 6-hydroxydopamine (6-OHDA), a neurotoxin for noradrenergic fibers, caused a significant decrease of BK binding sites. This observation also suggests the presence of B2R on noradrenergic bulbospinal neurons. Finally, a small but significant density of binding sites for [¹²⁵I]-Tyr⁸-BK was detected in the intermediolateral nucleus, the cell bodies and dendrites of pre-ganglionic sympathetic fibers (Lopes *et al.*, 1995). In all these studies, the pharmacological characterization of binding sites with different kinin agonists and

antagonists of B1R and B2R revealed the presence of only B2R in rodents under normal physiological conditions.

Immunocytochemical studies have shown the presence of B2R in rat cortex and cerebellum. Immunoreactivity for the B2R was present in all layers, especially on the soma of pyramidal cells and Purkinje cells in the cerebellum (Chen, 2000). The immunoreactivity for B2R in the spinal cord was described in a review (Couture and Lindsey, 2000) and confirmed the pattern observed by autoradiography in rat (Lopes *et al.*, 1995) and guinea pig (Lopes *et al.*, 1993a). In humans, immunoreactivity for B2R was observed in neurons of the hypothalamus, thalamus, caudate nucleus, cerebral cortex and brainstem (Bhoola, 1996; Raidoo *et al.*, 1996; Raidoo *et al.*, 1997).

B1R immunoreactivity was observed in the laminae I and II of normotensive Wistar rats without apparent pathology (Wotherspoon and Winter, 2000). Using retrograde labeling, B1R was identified by immunocytochemistry in nerve endings of type C- and A δ -fibers in sciatic nerves (Ma and Tian, 2001). However, these receptors are not functional.

Kinin B1R is known to participate in neuropathic pain mechanisms (Quintao *et al.*, 2008). Thus, increased levels of B1R mRNA or protein have been detected in the dorsal root ganglion and spinal cord after sciatic nerve constriction injury in rodents (Rashid *et al.*, 2004; Petcu *et al.*, 2008).

9. Kinins in the CNS

The localization of endogenous kinins in the CNS was difficult to determine because of their rapid metabolism, the presence of several peptidases and contamination from blood vessels. BK immunoreactivity (BK-LI) was observed particularly in the hypothalamus, periaqueductal gray nucleus, perirhinal cortex, cingulate gyrus, ventral portion of caudate-putamen and lateral septal area (Corrêa *et al.*, 1979). Unfortunately, these studies did not provide data on possible cross-reactivity with kininogens.

Using high-performance liquid chromatography (HPLC) and the technique of radio-immunological tests, BK was detected in the whole brain (0.6 pmol/g) and found at various concentrations in specific regions: hypothalamus > spinal cord = brainstem and the medulla > corpus striatum > hippocampus > cerebellum > cerebral cortex = midbrain (Perry and Snyder, 1984; Kariya *et al.*, 1985).

10. Action of kinins on body temperature

The central action of BK on body temperature comes from the relationship with prostaglandins. In fact, BK can release prostaglandins (McGiff *et al.*, 1976) and it is known that prostaglandins injected i.c.v. cause an increase in body temperature in cat (Milton and Wendlandt, 1970) and rabbit (Stitt, 1973). BK injected i.c.v. caused a dose-dependent increase in body temperature in rabbits and this effect was partially inhibited by paracetamol and indomethacin (inhibitors of prostaglandin synthesis) (Almeida e Silva and Pela, 1978). A decade later, (Rao and Bhattacharya,

1988) showed the same effect after i.c.v. injections of BK in rats and its inhibition by indomethacin. These authors also suggested the involvement of serotonin in the mechanisms underlying the onset of hyperthermia after central injection of BK. The physiological importance of these data is supported by the involvement of endogenous kinins in the hyperthermia induced by either central or systemic administration of LPS in rats (Walker *et al.*, 1996a,b; Coelho *et al.*, 1997). B2R and prostaglandins were involved in the early event of hyperthermia (Walker *et al.*, 1996a, b). Both B1R and B2R also participated in the induction and maintenance of fever induced by systemic injection of LPS (Coelho *et al.*, 1997). Destruction of the parvocellular region of the paraventricular nucleus of the hypothalamus with ibotenic acid significantly reduced fever induced by BK or by systemic injection of LPS, suggesting that this region is the site of action (Caldeira *et al.*, 1998) of BK.

Chapter II

Objectives

11. Objectives

11.1 First Objective

To investigate whether the tachykinin NK-3R in the VTA contributes to the maintenance of high arterial blood pressure through the mesocorticolimbic DA system in freely behaving SHR. This was accomplished by determining the cardiovascular response to selective NK-3R agonist and antagonists injected either into the i.c.v. or VTA before and after inhibition of DA-D1R and D2R or after destruction of the VTA with ibotenic acid. This work constitutes the first article given in Chapter III:

Blockade of tachykinin NK-3 receptor reverses hypertension through a dopaminergic mechanism in the ventral tegmental area of spontaneously hypertensive rats. British Journal of Pharmacology Aug 26th 2010 by De Brito Gariepy H. and Couture R. PMID: 20804497.

11.2 Second Objective

To investigate whether the anti-hypertensive effect of the kinin B1R antagonist (SSR240612) observed in glucose-fed rats (Dias *et al.*, 2007) can be reproduced in two rat models of hypertension: SHR and Ang II-treated rats. In order to find out if the anti-hypertensive effect is either centrally or peripherally mediated, SSR240612 was administered by gavage and i.c.v., and two kinin B1R antagonists (R-715 and R-954), which do not cross the BBB, were injected subcutaneously. As the second goal, we decided to assess the contribution of central DA in the effects of SSR240612.

This is a pharmacological approach using conscious unrestrained rats. This study constitutes the second article given in Chapter III:

Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension. *Neuropeptides* 44(2):191-198, by De Brito Gariepy H, Carayon P, Ferrari B, Couture R (2010).

11.3 Third Objective

This study was undertaken to determine whether the overexpressed B1R is functional in the CNS of hypertensive rats. Brain B1R was stimulated by i.c.v. injection of the selective kinin B1R agonist Sar[DPhe⁸][des-Arg⁹]BK and the effect on behavioural activity was measured in SHR and Ang II-treated rats. The mechanism was studied with a pharmacological approach using selective receptor antagonists or enzymatic inhibitors for SP, glutamate, nitric oxide (NO), DA-D1R and D2R. A molecular approach was used to measure the expression of B1R at mRNA level in selected brain regions related to behaviour and the mesolimbic dopaminergic system in SHR and Ang II-treated rats. This study constitutes the third article given in Chapter III:

Mechanism underlying behavioural activity induced by activation of brain kinin B1 receptor in hypertensive rats, by De Brito Gariepy H, Talbot S, Couture R (2010) (submitted to *British Journal of Pharmacology*).

Chapter III

Articles

12. Articles

12.1 Article #1

Contributions:

H. De Brito Gariepy, contributed by performing all the experiments, created the graphics, figures, and wrote the manuscript. R. Couture contributed in the redaction and correction of the manuscript.

Blockade of tachykinin NK₃ receptor reverses hypertension through a dopaminergic mechanism in the ventral tegmental area of spontaneously hypertensive rats

Helaine De Brito Garipey and Réjean Couture*

Département de Physiologie, Faculté de médecine, Université de Montréal,
C.P. 6128, succursale Centre-ville, Montréal, QC, Canada - H3C 3J7

Running title: Central interaction between tachykinins and dopamine

Article accepted in the British Journal of Pharmacology August 26th 2010.

*Author for correspondence:

Dr. Réjean Couture

Telephone: 1-514-343-7060

Fax: 1-514-343-2111

Summary

Background and purpose. Intracerebroventricularly (i.c.v.) injected tachykinin NK₃ receptor (R) antagonists normalized mean arterial blood pressure (MAP) in spontaneously hypertensive rats (SHR). This study was pursued to define the role played by NK₃R located on dopamine (DA) neurons of the ventral tegmental area (VTA) in the regulation of MAP in SHR.

Experimental approach. SHR (16 weeks) were implanted permanently with i.c.v. and/or VTA guide cannulae. Experiments were conducted 24 h after catheterization of the abdominal aorta to measure MAP and heart rate (HR) in freely behaving rats. Cardiovascular responses to i.c.v. or VTA injected NK₃R agonist (senktide) and antagonists (SB222200 and R-820) were measured before and after systemic administration of selective DA antagonists for D₁R (SCH23390), D₂R (raclopride) or non-selective D₂R (haloperidol) and after destruction of the VTA with ibotenic acid.

Key results. I.c.v. or VTA injected SB222200 and R-820 (500 pmol) evoked anti-hypertension, which was blocked by raclopride. Senktide (10, 25, 65 and 100 pmol) elicited greater increases of MAP and HR when injected in the VTA and the cardiovascular response was blocked by R-820, SCH23390 and haloperidol. VTA injected SB222200 prevented the pressor response to i.c.v. senktide, and *vice versa*, i.c.v. senktide prevented the anti-hypertension to VTA SB222200. Destruction of the VTA prevented the pressor response to i.c.v. senktide and the anti-hypertension to i.c.v. R-820.

Conclusions and implications. Tachykinin NK₃R in the VTA is implicated in the maintenance of hypertension by increasing midbrain DA transmission in SHR. Hence, this receptor may represent a therapeutic target in the treatment of hypertension.

Keywords. Dopamine, tachykinins, NK₃R, senktide, SB222200, R-820, ventral tegmental area, hypertension, spontaneously hypertensive rats

Abbreviations. ANOVA, analysis of variance; aCSF, artificial cerebrospinal fluid; BP, blood pressure; DA, dopamine; DMSO, dimethylsulphoxide; HR, heart rate; i.c.v., intracerebroventricular(ly); i.v., intravenous(ly); MAP, mean arterial blood pressure; NKA, neurokinin A; NKB, neurokinin B; NK₁R, tachykinin NK₁ receptor; NK₂R, tachykinin NK₂ receptor; NK₃R, tachykinin NK₃ receptor; s.c., subcutaneous(ly); SHR, spontaneously hypertensive rats; SP, substance P; WKY, Wistar Kyoto; VTA, ventral tegmental area.

Introduction

The ventral tegmental area (VTA), the origin of the mesolimbic and mesocortical (A10) dopaminergic (DA) pathways is traditionally known for its implication in the regulation of locomotor activity, stress responses, reinforcement and reward mechanisms (Le Moal and Simon, 1991; Swanson *et al.*, 2000; Hyman and Malenka, 2001). The literature from the 1990s suggests a role for the VTA and the mesolimbic DA system in cardiovascular homeostasis in link with the defence reaction and the somatosensory input (van den Buuse, 1998). The mesolimbic DA system is involved in diurnal blood pressure (BP) and heart rate (HR) regulation (Sei *et al.*, 1999; Sakata *et al.*, 2002). The discharge rate of DA neurons in the VTA seems to be regulated by inputs from arterial baroreceptors, likely through a relay in the *nucleus tractus solitarius* (Kirouac and Ciriello, 1997). Stimulation of the VTA is also associated with the modulation of the circulatory and pressor effects of vasopressin (van den Buuse and Catanzariti, 2000). Electrical stimulation of the rat VTA or microinjection of a substance P (SP) analogue produces increases of BP and HR through the activation of DA-D₁ and D₂ receptors (R), and vasopressin release (Cornish and van den Buuse, 1995).

In a further recent study, the VTA was proposed as a potential target for tachykinin receptors in cardiovascular regulation (Deschamps and Couture 2005). Increases of BP, HR and stereotypic behaviours are elicited by the intra VTA injection of physiological doses (pmol) of tachykinin NK₁, NK₂ and NK₃ receptor agonists in freely moving rats. These responses are

blocked in a selective manner by their respective antagonist and by systemic treatment with the DA-D₁R antagonist, SCH23390, but not with the DA-D₂R antagonist, raclopride. Furthermore, the cardiovascular response to tachykinin agonists is ascribed to enhanced cardiac output following the stimulation of cardiac β_1 -adrenoceptors. This study is consistent with the increased firing rate of A10 dopamine cells induced by application of NK₁, NK₂ and NK₃ receptors agonists in the VTA (Overton *et al.*, 1992) and the presence of NK₁ and NK₃ receptors on DA and non-DA neurons in the VTA as revealed by confocal and electron microscopy (Chen *et al.*, 1998; Lessard *et al.*, 2007; 2009). The VTA is enriched of NK₃R binding sites (Dam *et al.*, 1990; Stoessl and Hill, 1990; Ding *et al.*, 1996; Langlois *et al.*, 2001) and is particularly sensitive to the NK₃R agonist senktide (Seabrook *et al.*, 1995; Deschamps and Couture, 2005). SP and senktide injected into the VTA enhance levels of DA and its metabolite dihydroxyphenylacetic acid (DOPAC), DA turnover in the prefrontal cortex and nucleus accumbens (Elliott *et al.*, 1986; Cador *et al.*, 1989; Overton *et al.*, 1992; Marco *et al.*, 1998), and behaviour consistent with mesolimbic DA activation (Eison *et al.*, 1982; Elliott and Iversen, 1986; Deschamps and Couture, 2005). SP, neurokinins A and B are found in the VTA (Deutch *et al.*, 1985; Kalivas *et al.*, 1985; Warden and Young, 1988) and SP immunoreactive axon terminals make direct synaptic contact with DA neurons in the VTA (Tamiya *et al.*, 1990). SP projecting fibres in the VTA originate from the nucleus accumbens and the habenular nucleus (Cuello *et al.*, 1978; Lu *et al.*, 1998).

In a recent study, we reported that intracerebroventricular (i.c.v.) injection of NK₃R antagonists reverse hypertension in spontaneously hypertensive rats (SHR) without affecting resting blood pressure in Wistar-Kyoto rats (WKY) (Lessard *et al.*, 2004). Conversely, inhibition of brain NK₁R and NK₂R fails to affect blood pressure in SHR and WKY. Whereas these findings support a role for brain tachykinin NK₃R in the maintenance of hypertension in SHR, the underlying mechanism of the anti-hypertensive effect of NK₃R antagonists remains elusive. We showed that it persists after bilateral nephrectomy and is not accompanied of changes in plasma levels of vasopressin (Lessard *et al.*, 2004).

Based on all these revised findings, the VTA may represent a strategic site for the central anti-hypertensive effect of NK₃R antagonists in SHR. This is in keeping with the increased central DA activity which contributes substantially to hypertension in SHR (van den Buuse, 1997; Amenta *et al.*, 2001). An upregulation of D₁R and D₂R in the nucleus accumbens, one major neuronal projection of the VTA, was reported in young SHR (Kirouac and Ganguly, 1993; Vaughan *et al.*, 1999; Russell, 2003).

The present pharmacological study was undertaken to test the hypothesis that tachykinin NK₃R in the VTA contributes to the maintenance of high arterial blood pressure by interacting with the mesocorticolimbic DA system in freely behaving SHR. This was achieved by determining the cardiovascular response to selective NK₃R agonist (senktide) and antagonists (SB222200 and R-820) injected i.c.v. or into the VTA before and after inhibition of DA-D₁R and D₂R (with SCH23390 and raclopride,

respectively) or after destruction of the VTA with ibotenic acid. Data suggest that tachykinin NK₃R located in the VTA are subjected to persisting tonic activation which increases midbrain DA transmission and thereby contributes to hypertension in SHR.

Methods

Animal source and care

Male SHR (16 weeks) were purchased one week prior to experiments from Charles River (St. Constant, Qc, Canada). They were housed two per cage under a 12 h light-dark cycle in a room with controlled temperature (22°C) and humidity (40%). Food (Charles River Rodent) and tap water were available *ad libitum*. The care of animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Animal Care Committee of our University.

Animal preparation

Before each surgery, rats received the antibiotics Trimethoprim and Sulphadiazine (Tribrissen 24%, 30 mgkg⁻¹, s.c., Schering Canada Inc., Pointe Claire, Qc, Canada) and the analgesic Ketoprophen (Anafen, 10 mgkg⁻¹, s.c., Merial Canada Inc., Baie d'Urfé, Qc, Canada). They were anaesthetized with isoflurane (3%) and then positioned in a stereotaxic apparatus (David Kopf Instrumentation, Tujunga, CA, U.S.A.) with the incisor bar set at 0.0 mm the interaural line for i.c.v. and at 3.3 mm below the interaural line for VTA implantation. The skull was exposed, cleaned and a hole was drilled to implant a 23-gauge stainless-steel guide cannulae into the left (contralateral) or right (ipsilateral) lateral brain ventricle (coordinates: 0.6 mm posterior to the bregma, 1.3 mm lateral to the midline, 3.0 mm ventral from the skull surface) and/or 2 mm above the left or right VTA (coordinates: 5.3-5.6 mm posterior to the bregma, 0.7 mm lateral to the

midline, 7.0 mm ventral from the skull surface) according to Paxinos and Watson (1998). The guide cannulae was fixed with two screws and dental cement to the skull. Then the skin was replaced and sutured. Finally, stylet (31-gauge stainless-steel) was inserted into the guide cannulae to prevent loss of cerebrospinal fluid and to avoid its obstruction. Animals were housed in individual plastic cages (40 x 23 x 20 cm) in the same controlled conditions and allowed to recover.

Three days later, the animals were re-anaesthetized with isoflurane and two siliconized PE-10 catheters (one PE-10 connected to PE-60), filled with physiological saline and 100 i.u.ml⁻¹ heparin sodium salt, were inserted into the abdominal aorta through the left femoral artery for blood pressure recording and into the left femoral vein for drug administration, respectively. They were passed subcutaneously to emerge at the back of the neck and secured with tape. During the following days, the animals were observed closely. Those which lost more than 20% of their body weight or had clear signs of cerebral haemorrhage, atypical behaviour or weaknesses were killed with CO₂ inhalation. Experimental protocols were initiated 24-48 h after the last vascular surgery in conscious and freely moving rats (Deschamps and Couture, 2005). Successful VTA and i.c.v. implantation were confirmed in all rats used in the study after *post-mortem* examination.

Measurement of cardiovascular parameters

During all experiments, continuous direct recordings of arterial blood pressure (BP) and heart rate (HR) were made on a high-performance data acquisition system PowerLab 8/30 with LabChart Pro ML870P and ML228 Bridge Amp (ADInstruments Inc, Colorado Springs, CO, USA). Mean arterial blood pressure (MAP) was calculated from systolic (S) and diastolic (D) blood pressure values $((S - D) \div 3 + D = \text{MAP})$.

The cardiovascular response was measured 1 h after the rats were transported to an isolated and quiet testing room. Rats remained in their resident cage, but the top grid containing the food and tap water was removed prior to and up to 1h post-injection. When BP and HR were stable, a 31-gauge stainless-steel injector connected to a Hamilton microsyringe 5 μl via a PE-10 tubing catheter was inserted into the guide cannulae which was accessible without handling the rat. Five min later, injection was made i.c.v. or directly into the VTA of undisturbed, freely behaving rats. All solutions were freshly prepared and injected (volume of 1 μl i.c.v.; 0.5 μl VTA and 0.1 ml kg^{-1} i.v.) over a period of 1 min. The injector was removed from the guide cannulae 1 min after injection to prevent any possible leakage of the injectate.

Experimental Protocols

Cardiovascular effects of i.c.v. and VTA administered tachykinin NK₃R antagonists. This series of experiments aimed at determining the anti-hypertensive effect of centrally administered NK₃R antagonists (500 pmol).

SHR received by i.c.v. the selective tachykinin NK₃R antagonist (SB222200, n=8) (Sarau *et al.*, 2000) 1h after the vehicle (artificial cerebrospinal fluid or aCSF, n=8). The inactive enantiomer of SB222200 (SB222201, n=10) was also injected as control for specificity in a separate group of SHR. The selection of this dose of SB222200 was based on a previous dose-response curve which established that i.c.v. 500 pmol caused the optimal anti-hypertensive effect in SHR (Lessard and Couture, 2004). SHR implanted with a VTA cannulae received aCSF and 1h later SB222200 (500 pmol, n=5) for comparison purpose.

In two separate groups of SHR, R-820 (500 pmol) was injected either i.c.v. (n=5) or directly into the VTA (n=4) and the anti-hypertensive responses were measured for up to 3 days or until they were back to baseline values. The dose and duration of recording were based on a previous i.c.v. study achieved in SHR (Lessard *et al.*, 2004).

Effects of systemic treatments with dopamine D₁ and D₂ receptor antagonists against SB222200 and R-820. This series of experiments was designed to determine the contribution of DA and its receptors in the anti-hypertensive effects of NK₃R antagonists. SHR that have received SB222200 (500 pmol, 24h earlier) or R-820 (500 pmol, 72h earlier) by the i.c.v. or the VTA route, were given the DA-D₁R antagonist SCH23390 (0.2 mgkg⁻¹, i.v.) (Kirouac and Ciriello, 1997; Gioanni *et al.*, 1998) or the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v.) (Millan *et al.*, 1998) 30 min prior to a second injection of NK₃R antagonist. SB222200 and R-820 (500 pmol) were re-injected again

either 24h (SB222200) or 72h (R-820) later to assess the reversibility of any blockade observed in the presence of DA antagonist. A rat received only one DA antagonist and one NK₃R antagonist either i.c.v or into the VTA.

Chronic anti-hypertensive effect of i.c.v. R-820. SHR (n=4) received daily an i.c.v. injection of R-820 (500 pmol) to see whether its anti-hypertensive effect could be maintained for a period of 5 days. MAP and HR were measured at the peak time within the 5h post-injection every day. The recording was pursued up to two days after the interruption of the treatment.

Dose-response curves with the NK₃R agonist senktide after i.c.v. and VTA microinjection. Two groups of SHR were used on 4 consecutive days; the first group was implanted with an i.c.v. cannulae (n=6) and the second group with a VTA cannulae (n=6). They received i.c.v. or directly into the VTA a microinjection of aCSF followed by a dose of 10 pmol senktide 60 min later, and then increasing doses of 25, 65 and 100 pmol were administered on the following 3 days at 24 h intervals. This protocol and the doses selected were based on our previous studies in which the cardiovascular effects of i.c.v. and VTA injected senktide were characterized in normotensive rats (Cellier *et al.*, 1997; Deschamps and Couture, 2005).

Blockade of senktide by tachykinin NK₃R antagonist in the VTA. In this series of experiments, the dose of 25 pmol senktide was selected on the basis of its maximal cardiovascular response upon VTA injection. SHR (n=4) received a microinjection of aCSF into the VTA followed 60 min later by a single

dose of senktide (25 pmol). Twenty-four hours later, senktide was re-injected again 1h after the VTA injection of the tachykinin NK₃R antagonist R-820 (500 pmol) (Regoli *et al.*, 1994). The reversibility of the inhibition was tested 24 h later.

Effects of systemic treatments with dopamine D₁ and D₂ receptor antagonists against i.c.v. senktide. In this series of experiments, the dose of 65 pmol senktide was selected on the basis of its maximal pressor response upon i.c.v. injection. Three groups of SHR were initially injected i.c.v. with a single dose of senktide (65 pmol). One hour later, they received intravenous (i.v.) or subcutaneous (s.c.) injection of either the DA-D₁R antagonist SCH23390 (0.2 mgkg⁻¹, i.v. n=6), the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v. n=6) or the non-selective DA-D₂R antagonist haloperidol (10 mgkg⁻¹, s.c. n=4) (Nsimba *et al.*, 1997; Miyamoto *et al.*, 2005), followed 30 min later by senktide (65 pmol). The following day (24 h later) senktide (65 pmol) was re-injected alone to assess the reversibility of any blockade observed in the presence of antagonist on the preceding day. In one additional group of SHR (n=4), raclopride and SCH23390 were administered alone at 24h apart to assess their direct cardiovascular effects. A second group of SHR (n=4) served to evaluate the direct cardiovascular effect of haloperidol. Pilot experiments have shown that the cardiovascular response to i.c.v. senktide (65 pmol) is reproducible when two injections are given at 1h apart.

Blockade of i.c.v. senktide by SB222200 injected into the VTA of SHR and reciprocally blockade of VTA SB222200 by i.c.v. senktide. This experiment

aimed at determining, firstly, the contribution of VTA NK₃R in the i.c.v. pressor response to senktide, and secondly, the possibility that the anti-hypertensive effect of SB222200 injected into the VTA could be prevented by the ipsilateral i.c.v. injection of the agonist senktide. Rats received senktide (65 pmol, n=4) by i.c.v (right side). Two hours later, SB222200 (500 pmol) was injected into the ipsilateral VTA, and senktide was re-injected i.c.v. 1 h later to evaluate the inhibition of the pressor response to senktide. On the second day, SB222200 (500 pmol, n=4) was injected into the right VTA to assess its anti-hypertensive response. On the third day, senktide (65 pmol) was injected i.c.v. (right side) 1h after injection of SB222200 into the right VTA to see if it could block the anti-hypertensive effect of SB222200, which starts one-hour post-injection. In another group of rats (n=4), senktide was injected i.c.v. on the contralateral side (left side) of VTA injection of SB222200 (right side) and the rest of the protocol was similar to that described above.

Effect of ipsilateral and contralateral lesions of the VTA with ibotenic acid on the cardiovascular response to senktide and R-820. Ibotenic acid is proposed as an effective technique for producing circumscribed lesions when local, discrete brain lesions are desired. This method works reliably without apparent damage to passing or underlying fibers and without causing distant lesion effects in the brain in contrast to kainic acid, a similar excitatory amino acid toxin (Guldin and Markowitsch, 1981; 1982).

Senktide (65 pmol) was given i.c.v. in two groups of sham-operated rats (n=4) and in rats subjected to ipsilateral (n=4) or contralateral (n=4) lesions of the VTA with ibotenic acid (1 µg in 0.5 µl) injected directly into the VTA, 5 days earlier. Twenty four h after senktide injection, R-820 (500 pmol) was given i.c.v. in the four groups of SHR.

Histology

At the end of the experimental protocol, the rats were killed with CO₂ inhalation and then received immediately 0.5 µl of Evans Blue dye (Sigma-Aldrich Canada) in the VTA. The brains were removed and fixed with 10% (vv⁻¹) buffered formalin (Fisher Scientific Inc., ON, Canada) and 20% (wv⁻¹) sucrose until the piece was floating (around 2 weeks) on the surface (Carson, 1992). Coronal sections (40 µm, cut on a freezing microtome) were mounted on glass slides and stained with cresyl violet for histological examination of the microinjections site. The injection site was confirmed by the presence of a black spot in the VTA with no evidence of haemorrhage or necrosis (Figure 1).

Drugs and solutions

The drug target nomenclature conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2009). aCSF was purchased from Harvard Bioscience (MA, U.S.A.). Succinyl-[Asp⁶,MePhe⁸]-SP (6-11) (senktide) (MW: 842) was purchased from Bachem Bioscience Inc. (King of Prussia, PA, U.S.A.). Raclopride, haloperidol, ibotenic acid and heparin were purchased from Sigma-Aldrich Canada and SCH23390 from Tocris

(Ellisville, MO, U.S.A.). Haloperidol was dissolved in ethanol and dimethylsulphoxide (DMSO) and completed in saline (final solution contained 3% ethanol and 0.5% DMSO). SCH23390 and raclopride were dissolved in DMSO and completed in saline (final solutions contained 3% DMSO). R-820 (3-indolylcarbonyl-Hyp-Phg-N (Me)-Bzl) (MW: 624.6) was a kind gift of late Dr. Jean-Luc Fauchère (Research Institute Servier, Paris, France) (Regoli *et al.*, 1994). SB222200 [(S)-(-)-N-(α -ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide (MW: 686.7) and its inactive enantiomer SB222201 were a donation of Dr. Henry M. Sarau (GlaxoSmithKline, PA, U.S.A.) (Sarau *et al.*, 2000). They were dissolved in DMSO and aCSF mixed with 2-hydroxypropyl- β -cyclodextrin (Sigma-Aldrich Canada) to obtain the desired solution (final solutions contained less than 15% DMSO and 20% 2-hydroxypropyl- β -cyclodextrin).

Statistical analysis of data

Results are expressed as the means \pm s.e.m. of values obtained from (n) rats. Statistical analysis of data was performed with Graph-Pad Prism software. Time-course effects were analyzed for statistical significance by a two-way analysis of variance (ANOVA) followed by a Bonferroni's test for multiple comparisons. A one-way ANOVA followed by a *post hoc* Dunnett's test was used for multiple comparisons to the same control group or by a *post hoc* Bonferroni's test for comparison between groups (area under the curve (AUC)). Only probability values (P) less than 0.05 were considered to be statistically significant.

Results

Cardiovascular responses induced by i.c.v. or VTA injected SB222200

The tachykinin NK₃R antagonist SB222200 (500 pmol) injected i.c.v. caused a significant anti-hypertensive effect from 5 to 10 h post-injection in comparison with vehicle values in SHR. MAP was back to hypertensive levels (184.1 ± 3.8 mmHg) 24 h later (Figure 2a). The anti-hypertension induced by SB222200 peaked at 8 h (-42 ± 5.5 mmHg, $P < 0.001$) and was associated with a significant bradycardia. In contrast, the inactive enantiomer of SB222200 (SB222201) did not affect MAP or HR in comparison with vehicle values.

The injection of SB222200 (500 pmol) into the VTA caused a more rapid decrease in MAP, which was significant at 1h and peaked at 3 h post-injection in SHR. This anti-hypertensive effect of the NK₃R antagonist was resolved at 8h and was associated with a significant increase in HR at 3h and during the remaining recording period of 8h (Figure 2b).

Effects of dopamine antagonists on anti-hypertensive responses induced by tachykinin NK₃R antagonists.

Raclopride (0.16 mg kg^{-1} , i.v.) prevented the anti-hypertensive effect of i.c.v. SB222200 (500 pmol) and reduced the accompanied bradycardia (Figure 3a). Conversely, SCH23390 (0.2 mg kg^{-1} , i.v.) caused a small reduction of the cardiovascular response to SB222200 (Figure 3a). The anti-hypertensive effect mediated by i.c.v. SB222200 was completely recovered 24 h later when it was re-injected in the absence of DA antagonists (data not shown).

Likewise, the longer lasting anti-hypertensive response and bradycardia induced by i.c.v. R-820 (500 pmol) were blocked by raclopride (0.16 mgk⁻¹, i.v.) (Figure 3b). A similar treatment with raclopride also prevented the more rapid although shorter lasting anti-hypertensive response and the accompanied tachycardia elicited by R-820 injected into the VTA (Figure 4).

Chronic anti-hypertensive effect of i.c.v. R-820

In SHR, daily i.c.v. injection of R-820 (500 pmol) for 5 days led to a persistent decrease of both MAP and HR in comparison with the pre-administration values. MAP and HR values returned gradually to baseline hypertensive values within 48h after the interruption of the treatment with R-820 (Figure 5).

Comparison of the cardiovascular effect of i.c.v. and VTA injected senktide

The i.c.v. injections of the tachykinin NK₃R agonist senktide (10-100 pmol) increased significantly but not dose-dependently MAP and HR during the first 30-60 min post-injection in SHR. The pressor response was maximal at 65 pmol while the tachycardiac response was greater in intensity and duration at 25 pmol (Figure 6a).

When senktide (10-100 pmol) was injected directly into the VTA of SHR, it caused larger increases of MAP and HR than by i.c.v. (Figure 6b). These effects were, however, not dose-dependent and reached maximal values at 25 pmol for both parameters. The initial depressor response to senktide contributes to the smaller AUC for the MAP response at 65 pmol and may explain the absence of dose-dependent cardiovascular effect.

Effect of tachykinin NK₃R antagonist, R-820, on the cardiovascular response to senktide in the VTA

The increases of MAP and HR induced by senktide (25 pmol) injected into the VTA were completely abolished by the prior administration into the VTA of the NK₃R antagonist R-820 (500 pmol, 1h earlier) in SHR (Figure 7). The cardiovascular response to senktide was fully recovered 24 h later in the absence of R-820 (data not shown). When microinjection of senktide (25 pmol, n = 6) was made outside the VTA, no cardiovascular effect was observed. Also, no changes of blood pressure occurred after microinjection of R-820 (500 pmol, n = 8) outside the VTA (data not shown).

Effects of dopamine receptor antagonists on the pressor response induced by i.c.v. senktide

The dopamine D₁R antagonist SCH23390 (0.2 mgkg⁻¹, i.v., 30 min earlier) blocked the pressor effect mediated by i.c.v. senktide (65 pmol). In contrast, the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v., 30 min earlier) had a marginal effect on the pressor response induced by senktide (Figure 8 a, c). The same treatment with haloperidol (10 mgkg⁻¹, s.c., 30 min earlier), abolished the pressor effect caused by senktide (65 pmol) injected i.c.v. in SHR (Figure 8 b, c). The MAP response to senktide was completely recovered when it was re-injected alone 24 h after treatment with dopamine antagonists (data not shown).

At the dose used, the systemic administration of raclopride (0.16 mgkg⁻¹, i.v.) caused a transient increase of MAP, which was significant in

comparison with saline between 5 and 15 min post-injection. SCH23390 (0.2 mgkg⁻¹, i.v.) had no significant effect on MAP during the first hour post-injection. Conversely, haloperidol (10 mgkg⁻¹, s.c.) caused an anti-hypertensive effect which reaches significance from 1h up to 6h post-injection in comparison with saline (Figure 9). The maximal decrease in MAP induced by haloperidol at 3 h (-33 ± 5.5 mmHg, $P < 0.001$) was back to hypertensive levels 8 h later (184.1 ± 3.8 mmHg).

Interaction between i.c.v. senktide and VTA SB222200 in SHR. The pressor response evoked by i.c.v. senktide (65 pmol) was blocked when SB222200 (500 pmol) was injected into the ipsilateral VTA, 1h prior to a second injection of i.c.v. senktide (Figure 10 a,e). In contrast, when SB222200 was injected into the VTA under the same conditions, the pressor response to senktide injected i.c.v. on the contralateral side was little affected (Figure 10 b, f). Moreover, the anti-hypertensive effect which occurred from 1 to 5h after VTA injection of SB222200 (500 pmol) was blocked when senktide was injected i.c.v. on the ipsilateral side (Figure 10 c, e), yet it was only slightly reduced when senktide was injected i.c.v. on the contralateral side (Figure 10 d, f). This suggests that i.c.v. senktide diffused chiefly to the ipsilateral VTA to cause its pressor effect and to prevent the anti-hypertensive effect of SB222200. This also suggests that microinjected SB222200 into the VTA had limited diffusion to the contralateral VTA.

Effect of ipsilateral and contralateral lesions of the VTA with ibotenic acid on the cardiovascular response to senktide and R-820.

The pressor response induced by i.c.v. senktide (65 pmol) in sham-operated SHR was absent in rats subjected to ipsilateral lesion of the VTA with ibotenic acid (Figure 11 a, e). One day after the senktide experiment, R-820 (500 pmol) was given i.c.v. to both groups of rats. Only the sham-operated rats displayed the typical anti-hypertensive response to R-820 (Figure 11 c, e). Conversely, the pressor response induced by i.c.v. senktide (65 pmol) in sham-operated SHR was similar to that observed in rats subjected to contralateral lesion of the VTA with ibotenic acid (Figure 11 b, f). One day after the senktide experiment, the anti-hypertensive effect of i.c.v. R-820 (500 pmol) was very little affected by the contralateral lesion of the VTA (Figure 11 d, f).

Discussion

This study extended our earlier work showing that tachykinin NK₃R antagonists exert dose-dependent and reversible anti-hypertensive effects when injected intracerebrally in SHR (Lessard *et al.*, 2004). The present findings demonstrate that this anti-hypertension is mediated by the VTA and the mesocorticolimbic DA pathway. This statement is based on four arguments: 1- the anti-hypertensive effect of NK₃R antagonists injected i.c.v. was reproduced with a faster onset when drugs were injected directly into the VTA; 2- the cardiovascular responses elicited by i.c.v. or VTA administration of NK₃R agonist and antagonists were blocked by DA antagonists; 3- the i.c.v. pressor effect of the NK₃R agonist was prevented following inhibition of NK₃R in the ipsilateral VTA, and *vice versa*, the anti-hypertensive effect of SB222200 injected into the VTA was blocked by

ipsilateral i.c.v. injection of senktide; 4- chemical destruction of the ipsilateral VTA with ibotenic acid eliminated completely the cardiovascular response induced by i.c.v. senktide and R-820. Hence, these findings suggest that the VTA and its brain DA projections are of primary importance in the cardiovascular effects associated to i.c.v. injection of NK₃R agonist and antagonist.

I.c.v. injection of senktide increased HR and blood pressure in normotensive rat (Cellier *et al.*, 1997) and guinea-pig (Roccon *et al.*, 1996). This cardiovascular response was blocked by the prior i.c.v. injection of the NK₃R antagonist R-820 in normotensive rat (Cellier *et al.*, 1997) and was ascribed to the activation of the sympatho-adrenal system (Nagashima *et al.*, 1989; Roccon *et al.*, 1996). The cardiovascular response to senktide in the VTA, also blocked by R-820 (present study), was mediated by the sympathetic nervous system and the subsequent increase of cardiac output as it was abolished by i.v. treatment with atenolol, a β_1 -adrenoceptor antagonist (Deschamps and Couture, 2005). The latter study showed that the cardiovascular response to senktide in the VTA was similar to that caused by glutamic acid, which is consistent with neuronal cell activation. Moreover, the cardiovascular response to central injection of senktide in SHR is likely mediated by DA release because it was abolished after treatment with SCH23390 and haloperidol. The lack of effect of raclopride suggests the involvement of DA-D₁R rather than DA-D₂R, as reported previously in normotensive rats (Deschamps and Couture, 2005). This is

congruent with the release of DA in the nucleus accumbens following the activation of NK₃R by senktide in the VTA (Spooren *et al.*, 2005) and with the localization of NK₃R on DA neurons in the VTA (Chen *et al.*, 1998; Lessard *et al.*, 2007; 2009).

Findings suggest that tonic activation of NK₃R in the VTA enhances the release of DA in the mesocorticolimbic system and contributes to high blood pressure in SHR. This hypothesis is supported by the normalization of high blood pressure under blockade of NK₃R in the VTA through a mechanism sensitive to DA inhibition. The persisting anti-hypertensive effect under the 5-day i.c.v. administration of R-820 suggests that NK₃R is under constant and chronic tonic activation in SHR. By the same token, this experiment also substantiates the use of centrally acting NK₃R antagonists in the treatment of hypertension in this model. Since the anti-hypertensive effect of NK₃R antagonists was prevented by raclopride and much less by SCH23390, it is concluded that this effect is chiefly mediated by DA-D₂R. The suggestion of a greater basal release of tachykinins in the mesocorticolimbic system of SHR is consistent with the existence of endogenous tachykinins in the VTA (Kalivas *et al.*, 1985; Warden and Young, 1988; Tamiya *et al.*, 1990; Lu *et al.*, 1998) and the increased neurokinin B-like immunoreactive contents in the brain of SHR, the putative NK₃R endogenous ligand (Nagashima *et al.*, 1989). Since D₁R and D₂R are up-regulated in the nucleus accumbens in SHR (Kirouac and Ganguly, 1993; Vaughan *et al.*, 1999), it is tempting to suggest that the tonic

activation of NK₃R by endogenous tachykinins in the VTA is facilitated by a hyperactive DA mesolimbic system. Reciprocally, tonic activation of NK₃R may contribute to the hyperactivity of the VTA DA neuronal pathway and thereby in the maintenance of hypertension in SHR. The affinity and densities of specific NK₃R binding sites measured in the VTA and other midbrain nuclei were, however, not significantly different between SHR and WKY (Lessard *et al.*, 2003).

Because i.c.v. injection of NK₃R antagonists had no significant effect on resting blood pressure in normotensive rats, it was concluded that brain NK₃R did not exert a tonic autonomic control of blood pressure in normotensive rats (Deschamps and Couture, 2005; Couture *et al.*, 1995; Cellier *et al.*, 1997; Lessard *et al.*, 2004). Nevertheless, the mesolimbic DA system is involved in diurnal BP and HR regulation (Sei *et al.*, 1999; Sakata *et al.*, 2002) and therefore, the VTA may represent an important site of action for tachykinins in cardiovascular regulation in normotensive subject.

Whereas SB222200 and R-820 caused a tachycardia when injected into the VTA, both antagonists induced a small bradycardia at the peak of the anti-hypertensive response upon i.c.v. injection. The occurrence of tachycardia in response to a fall of blood pressure suggests that the inhibition of NK₃R in the VTA did not interfere with the activation of baroreceptors contrarily to i.c.v. administration of NK₃R antagonists and further suggests that inhibition of the cardiac output or baroreflex activity is unlikely the mechanism of the anti-hypertension. Although the increase of blood

pressure and HR induced by i.c.v. and VTA injected senktide was ascribed to the activation of the sympathetic nervous system (Roccon *et al.*, 1996; Deschamps and Couture, 2005), the decrease of blood pressure induced by i.c.v. injected SB222200 was not associated with changes in plasma catecholamines levels in SHR (Lessard *et al.*, 2004). One cannot exclude, however, that central tachykinin NK₃R antagonists may exert inhibitory action on neuronal sympathetic vasomotor tone.

The dissimilar time-course of the anti-hypertensive effect of R-820 and SB222200 was also observed in our previous study (Lessard *et al.*, 2004) and may be explained by the different physico-chemical features of these two compounds. SB222200, a much more lipophilic molecule than R-820, can diffuse more easily into the brain after i.c.v. injection. This could explain why SB222200 displays a quicker but less prolonged effect than R-820. Peripheral administration of these antagonists at 500 pmol in SHR had no effect on blood pressure, supporting a central site of action (Lessard *et al.*, 2004) that has been identified in the present study as the VTA.

The anti-hypertensive effect of s.c. administered haloperidol in SHR was not shared by selective blockade of D₁R and D₂R, which caused either no effect or a transient pressor response. The non-selectivity of haloperidol versus SCH23390 and raclopride for DA receptors and the putative inhibition of non DA receptors can account for the pronounced anti-hypertensive response of haloperidol. Although data of the present study obtained with haloperidol support the conclusion drawn with the selective

DA antagonist SCH23390 on the cardiovascular effect of senktide, they must be cautiously interpreted. The reduction of mean arterial blood pressure with haloperidol was also observed in angiotensin II-hypertensive rats while the same treatment had no effect in normotensive rats (De Brito Gariepy *et al.*, 2010). Haloperidol was suggested as treatment of hypertensive crises caused by high-dose amphetamine or methamphetamine abuse (Angrist *et al.*, 2001).

Conclusion

This neuropharmacological study suggests that tachykinin NK₃R located in the VTA are subjected to persisting tonic activation which increases midbrain DA transmission and thereby contributes to hypertension in SHR. Hence, these receptors may represent a therapeutic target in the treatment of arterial hypertension. Since NK₃R antagonists are proposed as putative antipsychotics in the treatment of schizophrenia (Spooren *et al.*, 2005; Meltzer and Prus, 2006), understanding the functional interaction between NK₃R and the DA system in the central autonomic control of blood pressure is of prime importance.

Acknowledgements

This work was supported by a Grant-in-aid from the Canadian Institutes of Health Research (MOP-79471). H. De Brito Gariepy held a Studentship from the National Council of Technological and Scientific Development (CNPq) (proc. 201428/2003-2) Brazil. We are grateful to Ms Paula Jansen Santana for the Artwork of Figure 1.

Statement of conflicts of interest

None.

References

Alexander SPH, Mathie A, Peters JA (2009). Guide to Receptors and Channels (GRAC), 4th edition. *Br J Pharmacol* **158 (Suppl. 1)**: S1-S254.

Amenta F, Ricci A, Rossodivita I, Avola R, Tayebati SK (2001). The dopaminergic system in hypertension. *Clin Exp Hypertension* **23 (1&2)**: 15-24.

Angrist B, Sanfilipo M, Wolkin A (2001). Cardiovascular effects of 0.5 milligrams per kilogram oral d-amphetamine and possible attenuation by haloperidol. *Clin Neuropharmacol* **24 (3)**: 139-144.

Cador M, Rivet JM, Kelley AE, Le Moal M, Stinus L (1989). Substance P, neurotensin and enkephalin injections into the ventral tegmental area: comparative study on dopamine turnover in several forebrain structures. *Brain Res* **486**: 357-363.

Carson R. (1992). Histotechnology: A Self-Instructional Text, 1st Ed. pp12-13, ASCP Press.

Cellier E, Barbot L, Regoli D, Couture R (1997). Cardiovascular and behavioural effects of intracerebroventricularly administered tachykinin NK3 receptor antagonists in the conscious rat. *Br J Pharmacol* **122 (4)**: 643-54.

Chen LW, Guan ZL, Ding YQ (1998). Mesencephalic dopaminergic neurons expressing neuromedin K receptor (NK3): a double immunocytochemical study in the rat. *Brain Res* **780**: 148-152.

Cornish JL, Van den Buuse M (1995). Stimulation of the rat mesolimbic dopaminergic system produces a pressor response which is mediated by dopamine D1 and D2 receptor activation and the release of vasopressin. *Brain Res* 701 (1-2): 28-38.

Couture R, Picard P, Poulat P, Prat A (1995). Characterization of the tachykinin receptors involved in spinal and supraspinal cardiovascular regulation. *Can J Physiol Pharmacol* 73 (7): 892-902.

Cuello AC, Emson PC, Paxinos G, Jessell T (1978) Substance P containing and cholinergic projections from the habenula. *Brain Res* 149: 413-429.

Dam T-V, Escher E, Quirion R (1990). Visualization of neurokinin-3 receptor sites in rat brain using the highly selective ligand [³H] senktide. *Brain Res* 506: 175-179.

De Brito Gariepy H, Carayon P, Ferrari B, Couture R (2010). Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension. *Neuropeptides* 44: 191-198.

Deschamps K, Couture R (2005). The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br J Pharmacol* 145 (6): 712-727.

Deutch AY, Maggio JE, Bannon MJ, Kalivas PW, Tam SY, Goldstein M, Roth RH (1985). Substance K and substance P differentially modulate mesolimbic and mesocortical systems. *Peptides* 6 (Suppl 2): 113-122.

Ding YQ, Shigemoto R, Takada M, Ohishi H, Nakanishi S, Mizuno N (1996). Localization of the neuromedin K receptor (NK₃) in the central nervous system of the rat. *J Comp Neurol* 364: 290-310.

Eison AS, Eison MS, Iversen SD (1982). The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area or substantia nigra of rat brain. *Brain Res* **238 (1)**: 137-52.

Elliott PJ, Alpert JE, Bannon MJ, Iversen SD (1986). Selective activation of mesolimbic and mesocortical dopamine metabolism in rat brain by infusion of a stable substance P analogue into the ventral tegmental area. *Brain Res* 363: 145-147.

Elliott PJ, Iversen SD (1986). Behavioural effects of tachykinins and related peptides. *Brain Res* 381: 68-76.

Gioanni Y, Thierry AM, Glowinski J, Tassin JP (1998). Alpha1-adrenergic, D1, and D2 receptors interactions in the prefrontal cortex: implications for the modality of action of different types of neuroleptics. *Synapse* **30 (4)**: 362-70.

Guldin WO, Markowitsch HJ (1981). No detectable remote lesions following massive intrastriatal injections of ibotenic acid. *Brain Res* **225**: 446-451.

Guldin WO, Markowitsch HJ (1982). Epidural kainate, but not ibotenate, produces lesions in local and distant regions of the brain. A comparison of the intracerebral actions of kainic acid and ibotenic acid. *J. Neurosci Methods* **5**: 83-93.

Hyman SE, Malenka RC (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat Rev Neurosci* **2**: 695-703.

Kalivas PW, Deutch AY, Maggio JE, Mantyh PW, Roth RH (1985). Substance K and substance P in the ventral tegmental area. *Neurosci Lett* **57(3)**: 241-246.

Kirouac GJ, Ganguly PK (1993). Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: an autoradiographic analysis. *Neuroscience* 52 (1):135-141.

Kirouac GJ, Ciriello J (1997). Cardiovascular depressor responses to stimulation of substantia nigra and ventral tegmental area. *Am J Physiol Heart Circ Physiol* 273: H2549-H2557.

Langlois X, Wintmolders C, Te Riele P, Leysen JE, Jurzak M (2001). Detailed distribution of neurokinin-3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. *Neuropharmacology* 40: 242-253.

Le Moal M, Simon H (1991). Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol Rev* 71 (1): 155-234.

Lessard A, Campos MM, Neugebauer W, Couture R (2003). Implication of nigral tachykinin NK3 receptors in the maintenance of hypertension in spontaneously hypertensive rats: a pharmacologic and autoradiographic study. *Br J Pharmacol* 138: 554-563.

Lessard A, Laurin M, Yamaguchi N, Couture R (2004). Central anti-hypertensive effect of tachykinin NK3 receptor antagonists in rat. *Eur J Pharmacol* 486 (1): 75-83.

Lessard A, Grady EF, Bunnett NW, Pickel VM (2007). Predominant surface distribution of neurokinin-3 receptors in non-dopaminergic dendrites in the rat substantia nigra and ventral tegmental area. *Neuroscience* 144 (4): 1393-1408.

Lessard A, Savard M, Gobeil F Jr, Pierce JP, Pickel VM. (2009). The neurokinin-3 (NK3) and the neurokinin-1 (NK1) receptors are differentially targeted to mesocortical and mesolimbic projection neurons and to neuronal nuclei in the rat ventral tegmental area. *Synapse* 63 (6):484-501.

Lu XY, Ghasemzadeh MB, Kalivas PW (1998). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* **82**: 767-780.

Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P, Steinberg R (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: an in vivo microdialysis approach in guinea pig. *Neuropeptides* **32 (5)**: 481-488.

Meltzer H, Prus A (2006). NK3 receptor antagonists for the treatment of schizophrenia. *Drug Disc Today Ther Strat* **3**: 555-560.

Millan MJ, Newman-Tancredi A, Brocco M, Gobert A, Lejeune F, Audinot V, Rivet JM, Schreiber R, Dekeyne A, Spedding M, Nicolas JP, Peglion JL (1998). S 18126 ([2-[4-(2, 3-dihydrobenzo[1, 4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D₄ receptors: an *in vitro* and *in vivo* comparison with L 745, 870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. *J Pharmacol Exp Ther* **287**:167-186.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA (2005). Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry* **10**: 79-104.

Nagashima A, Takano Y, Tateishi K, Matsuoka Y, Hamaoka T, Kamiya H. (1989). Central pressor actions of neurokinin B: increases in neurokinin B contents in discrete nuclei in spontaneously hypertensive rats. *Brain Res* **499 (1)**: 198-203.

Nsimba SED, Kelly JP, Leonard BE (1997). Effect of acute and chronic haloperidol administration and apomorphine challenge on the behavioural parameters in rats. *Indian J Pharmacol* **29** (1): 15-19.

Overton P, Elliott PJ, Hagan RM, Clark D (1992). Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. *Eur J Pharmacol* **213**: 165-166.

Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; San Diego, CA: 1998.

Regoli D, Boudon A, Fauchere JL (1994). Receptors and antagonists for substance P and related peptides. *Pharmacol Rev* **46** (4): 551-599.

Roccon A, Marchionni D, Nisato D (1996). Study of SR 142801, a new potent non-peptide NK3 receptor antagonist on cardiovascular responses in conscious guinea-pig. *Br J Pharmacol* **118** (5): 1095-102.

Russell VA (2003). Dopamine hypofunction possibly results from a defect in glutamate-stimulated release of dopamine in the nucleus accumbens shell of a rat model for attention deficit hyperactivity disorder-the spontaneously hypertensive rat. *Neurosci Biobehav Rev* **27** (7): 671-682.

Sakata M, Sei H, Toida K, Fujihara H, Urushihara R, Morita Y (2002). Mesolimbic dopaminergic system is involved in diurnal blood pressure regulation. *Brain Res* **928**: 194-201.

Sarau HM, Griswold DE, Bush B, Potts W, Sandhu P, Lundberg D, Foley JJ, Schmidt DB, Webb EF, Martin LD, Legos JJ, Whitmore RG, Barone FC, Medhurst AD, Luttmann MA, Giardina GA, Hay DW (2000). Nonpeptide tachykinin receptor antagonists. II. Pharmacological and pharmacokinetic profile of SB-222200, a central nervous system penetrant, potent and selective NK3 receptor antagonist. *J Pharmacol Exp Ther* **295** (1): 373-381.

Seabrook GR, Patel S, Hutson PH, Ragan CI, Bristow LJ (1995). Functional relevance of dopamine receptors: Further studies are required to determine the physiological role and therapeutic significance of dopamine D3 receptors. *Trends in Pharmacol Sci* 16: 406-408.

Sei H, Ikemoto K, Arai R, Morita Y (1999). Injection of 6-hydroxydopamine into the ventral tegmental area suppresses the increase in arterial pressure during REM sleep in the rat. *Sleep Res* 2 (1): 1-6.

Spooren W, Riemer C, Meltzer H (2005). NK3 receptor antagonists: the next generation of antipsychotics? *Nature Reviews Drug Discovery* 4: 967-975.

Stoessl AJ, Hill DR (1990). Autoradiographic visualization of NK-3 tachykinin binding sites in the rat brain, utilizing [3H]senktide. *Brain Res* 534 (1-2):1-7.

Swanson JM, Flodman P, Kennedy J, Spence MA, Moyziz R, Schuck S, Murias M, Moriarity J, Barr C, Smith M, Posner M (2000). Dopamine genes and ADHD. *Neurosci Biobehav Rev* 24 (1): 21-25.

Tamiya R, Hanada M, Kawai Y, Inagaki S, Takagi H (1990). Substance P afferents have synaptic contacts with dopaminergic neurons in the ventral tegmental area of the rat. *Neurosci Lett* 110 (1-2): 11-15.

Van den Buuse M (1997). Pressor responses to brain dopaminergic stimulation. *Clin Exp Pharmacol Physiol Rev* 24 (9-10): 764-769.

Van den Buuse M (1998). Role of the mesolimbic dopamine system in cardiovascular homeostasis. Stimulation of the ventral tegmental area modulates the effect of vasopressin on blood pressure in conscious rats. *Clin Exp Pharmacol Physiol* 25: 661-668.

Van den Buuse M, Catanzariti R (2000). Stimulation of the ventral tegmental area enhances the effect of vasopressin on blood pressure in conscious rats. *Br J Pharmacol* 129: 29-36.

Vaughan CE, Van den Buuse M, Roland BL (1999). Brain dopamine D2 receptor mRNA levels are elevated in young spontaneously hypertensive rats. *Neurosci Res* **34**: 199-205.

Warden MK, Young WS 3rd (1988). Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *J Comp Neurol* **272** (1): 90-113.

Figure Legends:

Figure 1: Identification of the VTA as microinjection site following *post-mortem* histological examination of microinjected Evans's blue. A rat was considered to be correctly injected when a black spot was seen in the VTA without any evidence of haemorrhage or necrosis. Diagram was modified from the atlas of Paxinos and Watson (1998). Abbreviations: VTA, ventral tegmental area; SNR, substantia nigra reticular; ml, medial lemniscus; RMC, red nucleus magnocellular; MS, microinjection site. Scale: 0.5 mm.

Figure 2: Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. (a) or VTA (b) injection of 500 pmol of SB222200 or its inactive enantiomer SB222201. Areas under the curves (AUC) were measured for a period of 0-10 h for i.c.v. and 0-8 h for VTA (small insets). Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF is indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 3: Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. injection of (a) SB222200 (500 pmol) and (b) R-820 (500 pmol) before and 30 min after treatment with the DA-D₂R antagonist raclopride (0.16 mgkg⁻¹, i.v.) or the DA -D₁R antagonist SCH23390 (0.2 mgkg⁻¹, i.v.). Areas under the curves (AUC) were measured for a period of 0-8 h (SB222200) or 0-72 h (R-820) as shown in small insets. Values represent the mean \pm s.e.m. of n rats. Statistical

comparison to aCSF values (*) or NK₃R antagonist alone (†) is indicated by *P<0.05, **†P<0.01; ***††P<0.001.

Figure 4: Time-course effects on changes in mean arterial blood pressure (Δ MAP) (a) and heart rate (Δ H) (b) induced by R-820 (500 pmol) injected into the VTA before and 30 min after treatment with the DA-D₂R antagonist raclopride (0.16 mg/kg⁻¹, i.v.). Areas under the curves (AUC) were measured for a period of 0-5 h (small insets). Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF values (*) or R-820 (†) is indicated by *†P<0.05; **††P<0.01; ***†††P<0.001.

Figure 5: Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) induced by i.c.v. injection of 500 pmol R-820, once daily for a period of 5 days in SHR. Values represent the mean \pm s.e.m. of 4 rats. Statistical comparison to pre-injection values is indicated by *P<0.05; **P<0.01.

Figure 6: Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) following i.c.v. (a) or VTA (b) injection of four increasing doses (10, 25, 65 and 100 pmol) of senktide in SHR. Areas under the curves (AUC) were measured for a period of 0-1 h (open bars for VTA and closed bars for i.c.v.). Values represent the mean \pm s.e.m. of 6 rats per injection site. Statistical comparison to aCSF values (*) or i.c.v. (†) is indicated by **††P<0.01; ***†††P<0.001.

Figure 7: Time-course effects on changes in mean arterial blood pressure (Δ MAP) and heart rate (Δ HR) produced by senktide (25 pmol) injected into the VTA prior to (first day) and 1 h after VTA injection of R-820 (second day). Each point represents the means \pm s.e.m. of 4 rats. Statistical comparison to aCSF (*) or senktide (†) values is indicated by *†P<0.05; **††P<0.01; ***†††P<0.001.

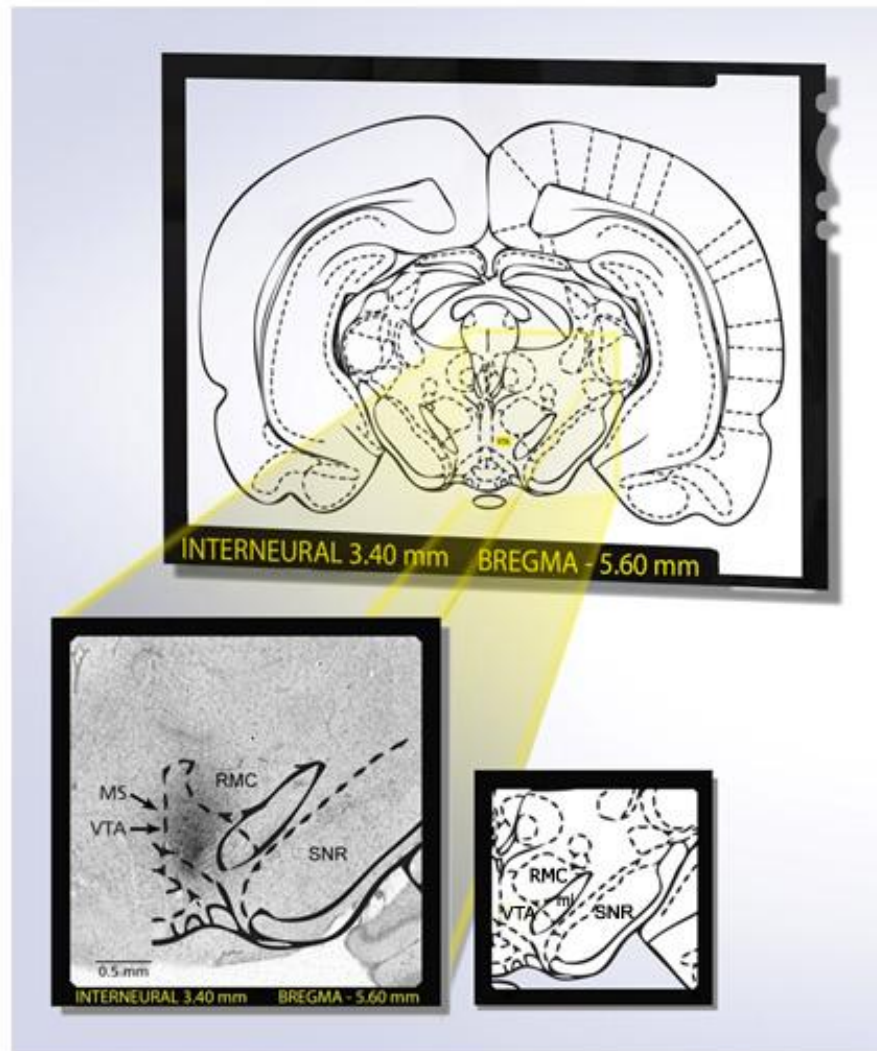
Figure 8: Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by i.c.v. senktide 65 pmol (baseline: 161 ± 5 mmHg) prior to and after treatment with (a) the DA-D₁R antagonist SCH23390 (0.2 mgkg^{-1} i.v.) and the DA-D₂R antagonist raclopride (0.16 mgkg^{-1} i.v.) (baseline: 167 ± 4 mmHg); and (b) the non-selective DA-D₂R antagonist haloperidol (10 mgkg^{-1} s.c.) (baseline: 164 ± 2.4 mmHg) in SHR. In (c) are shown the Areas under the curves (AUC) measured for a period of 0-1 h. Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF (*) or senktide (†) values is indicated by * †P<0.05; ** ††P<0.01; *** †††P<0.001.

Figure 9: Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by treatments with the DA-D₁R antagonist SCH23390 (0.2 mgkg^{-1} i.v.), DA-D₂R antagonist raclopride (0.16 mgkg^{-1} i.v.) and the non-selective DA-D₂R antagonist haloperidol (10 mgkg^{-1} s.c.) in SHR. Different time scales were used: in (a) (min), and (b) (hours). Values represent the mean \pm s.e.m. of 4 rats. Statistical comparison to saline is indicated by *P<0.05; **P<0.01; ***P<0.001.

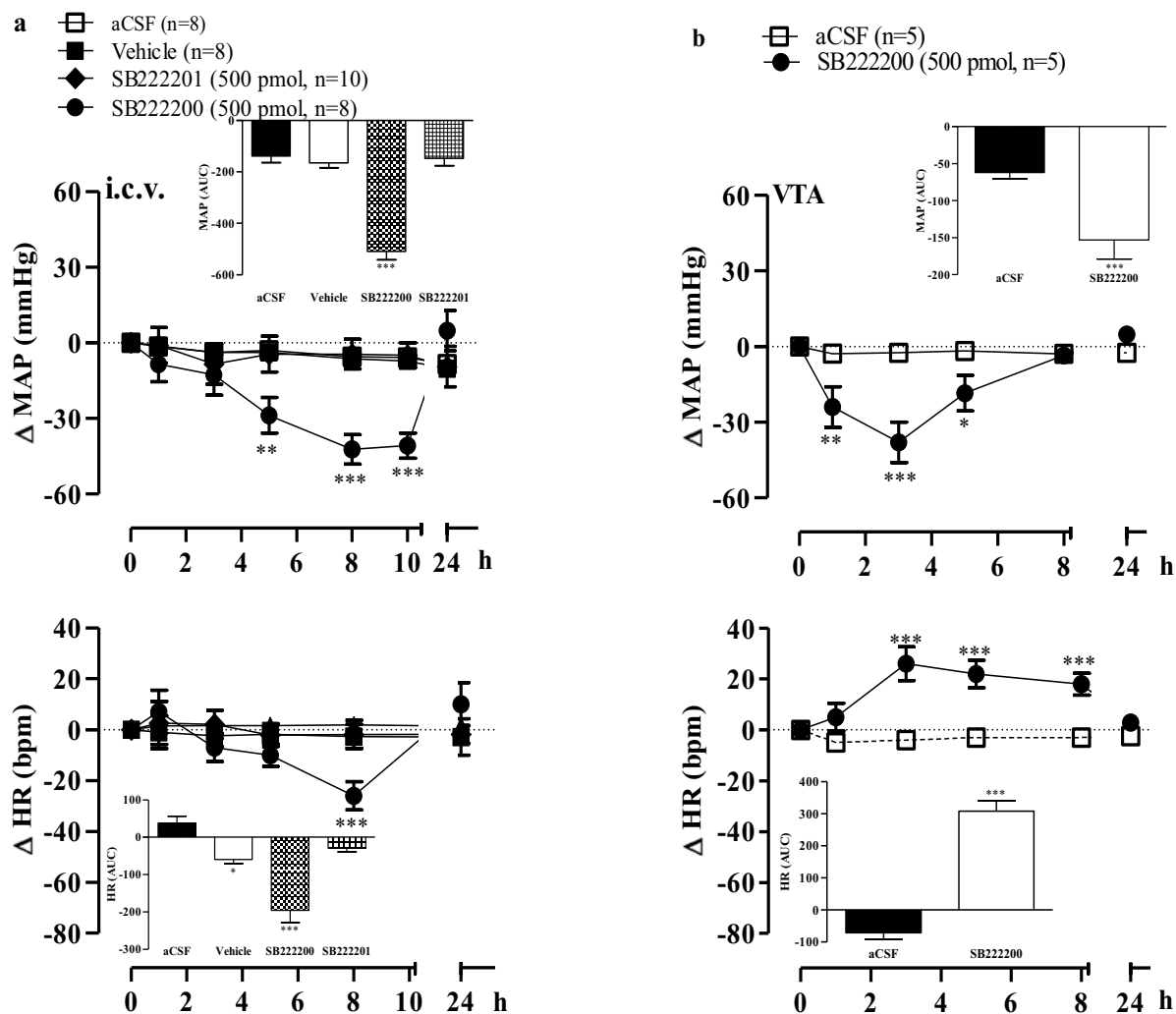
Figure 10: Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by senktide injected i.c.v. on the (a) ipsilateral and (b) contralateral side before and after SB222200 (500 pmol) injected into the VTA. The anti-hypertensive effect elicited by the VTA injection of SB222200 (500 pmol) was measured before and after i.c.v. injection of senktide (65 pmol) on the ipsilateral (c) and contralateral (d) side in SHR. Areas under the curves (AUC) for MAP are shown in (e and f). Values represent the mean \pm s.e.m. of n rats. Statistical comparison to aCSF (*) and senktide alone or SB222200 alone (†) is indicated by * † P<0.05; ** ††P<0.01; *** ††† P<0.001.

Figure 11: Time-course effects on changes in mean arterial blood pressure (Δ MAP) produced by i.c.v. injected 65 pmol senktide (a, b) or 500 pmol R-820 (c, d) in SHR which underwent an ipsilateral (a, c) and contralateral (b,

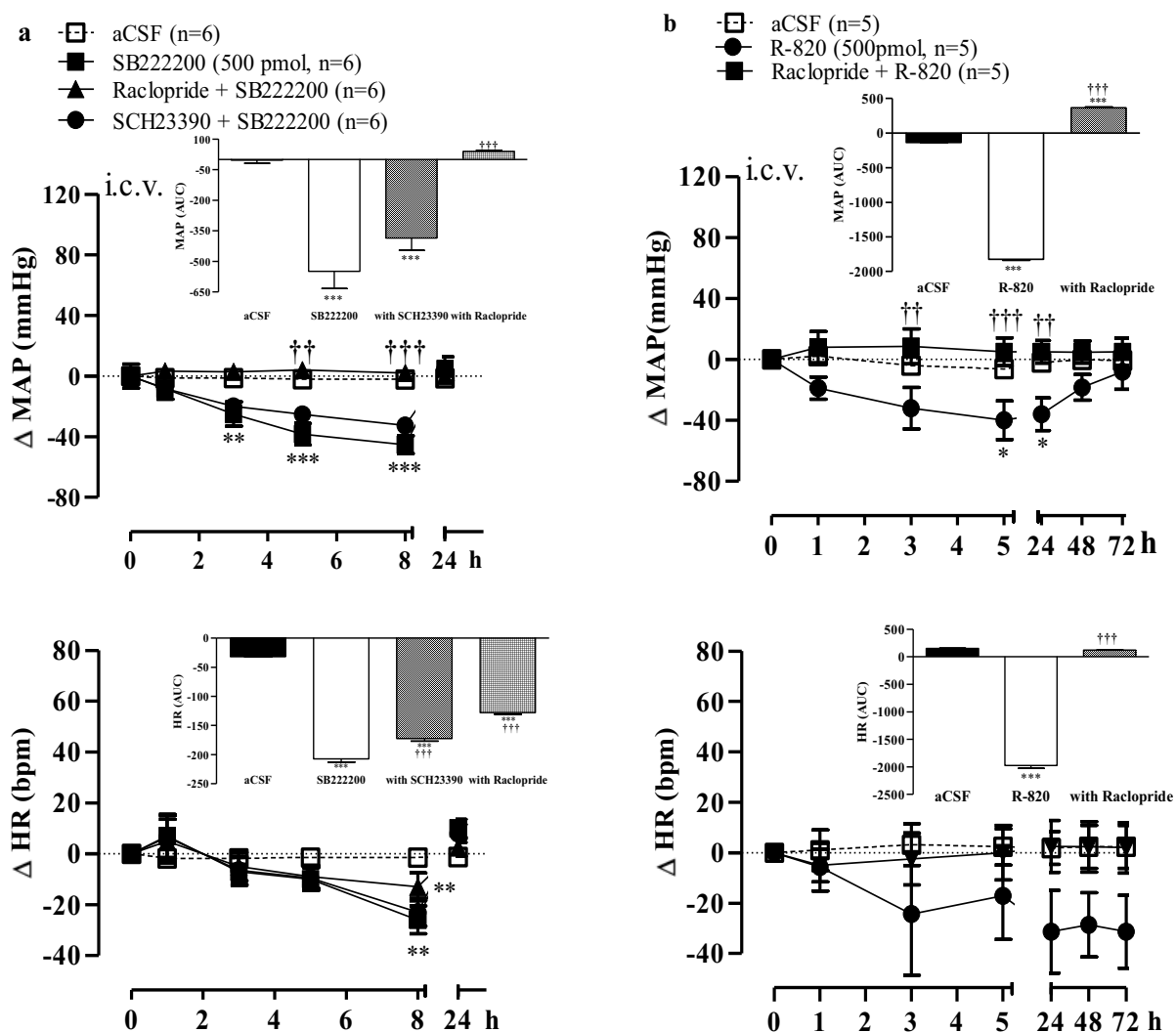
d) lesion of the VTA with ibotenic acid (IBO), 5 days earlier or a sham-operation. Areas under the curves (AUC) for MAP are shown in (e, f). Each point represents the mean \pm s.e.m of n rats. Statistical comparison to aCSF (*) or sham-operated rats (†) is indicated by * †P<0.05; ** ††P<0.01; *** †††P<0.001.



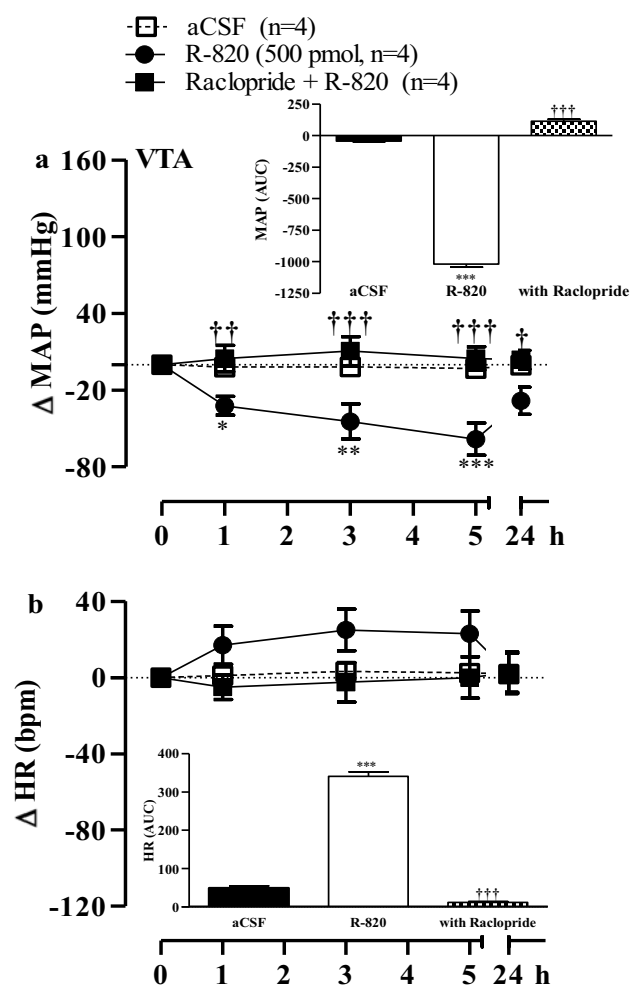
De Brito Garipey et al.
Figure 1

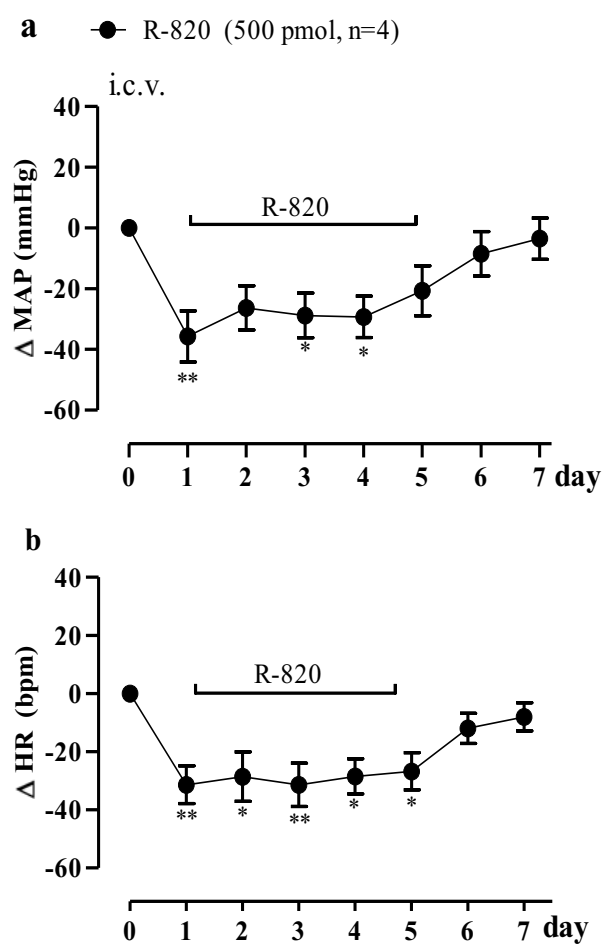


De Brito Gariepy et al.
Figure 2

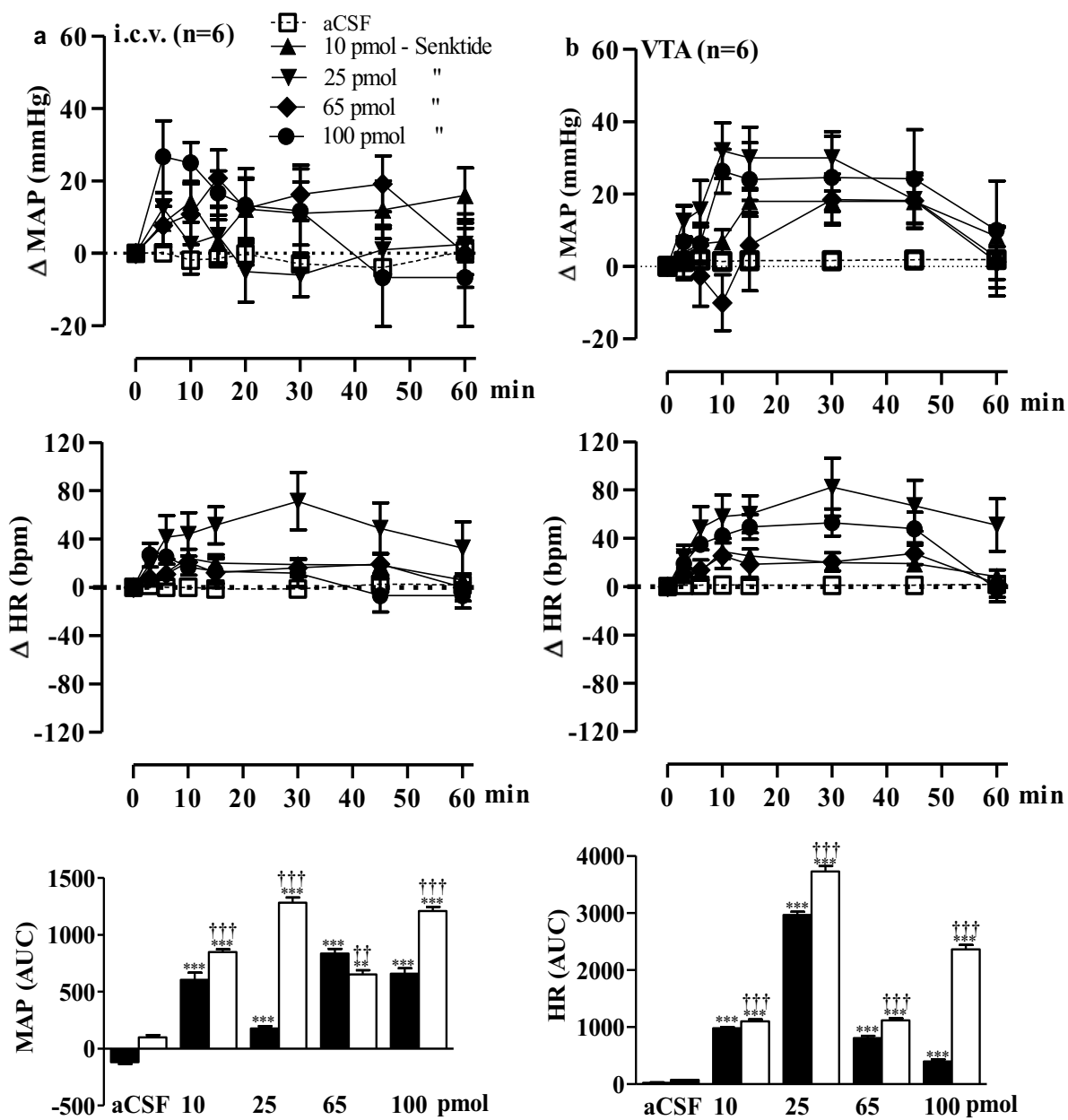


De Brito Gariepy et al.
Figure 3

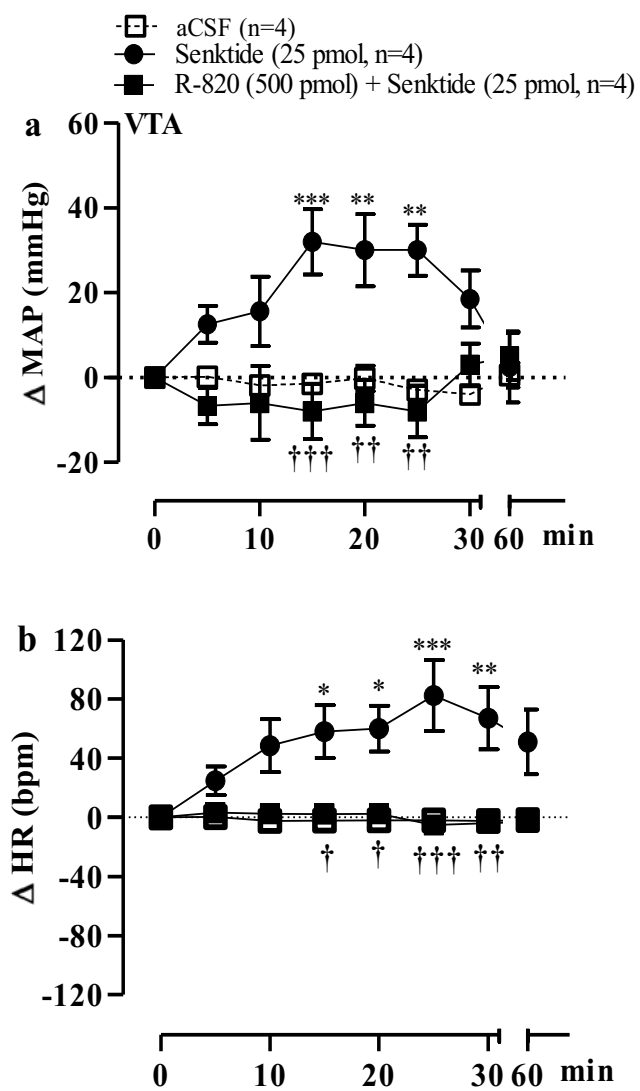




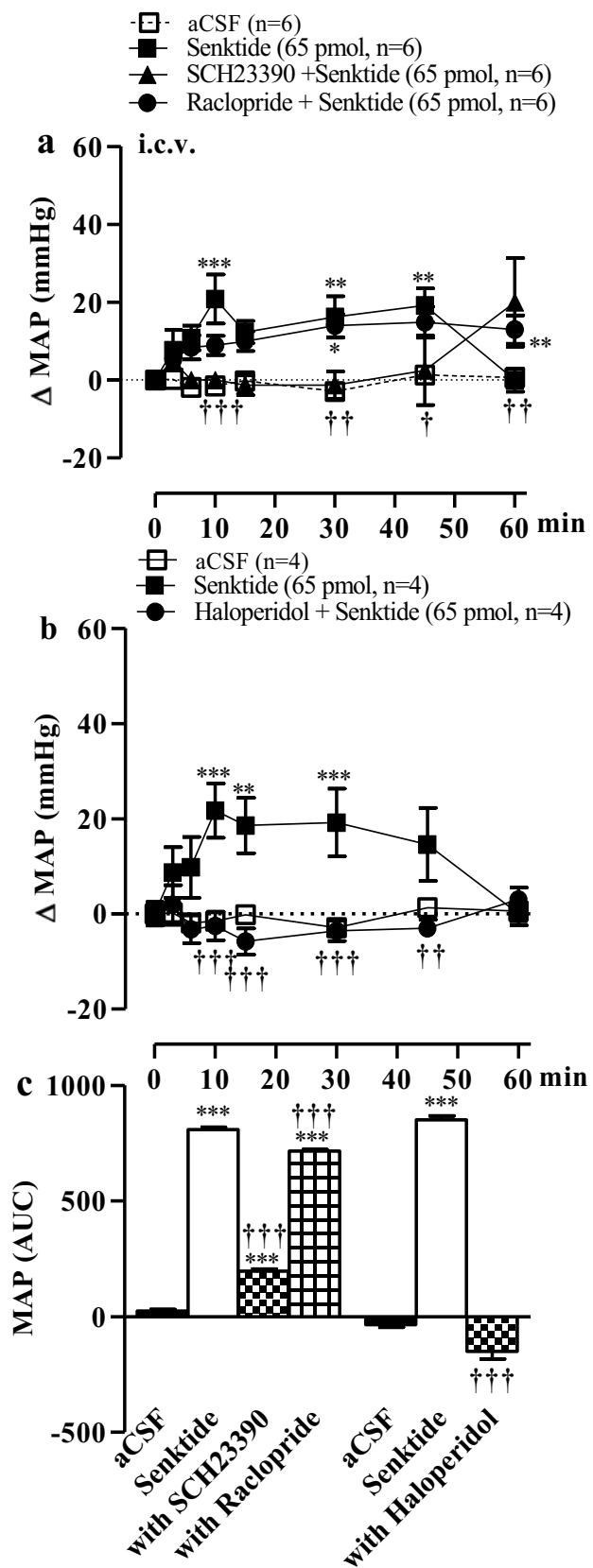
De Brito Garipey et al.
Figure 5



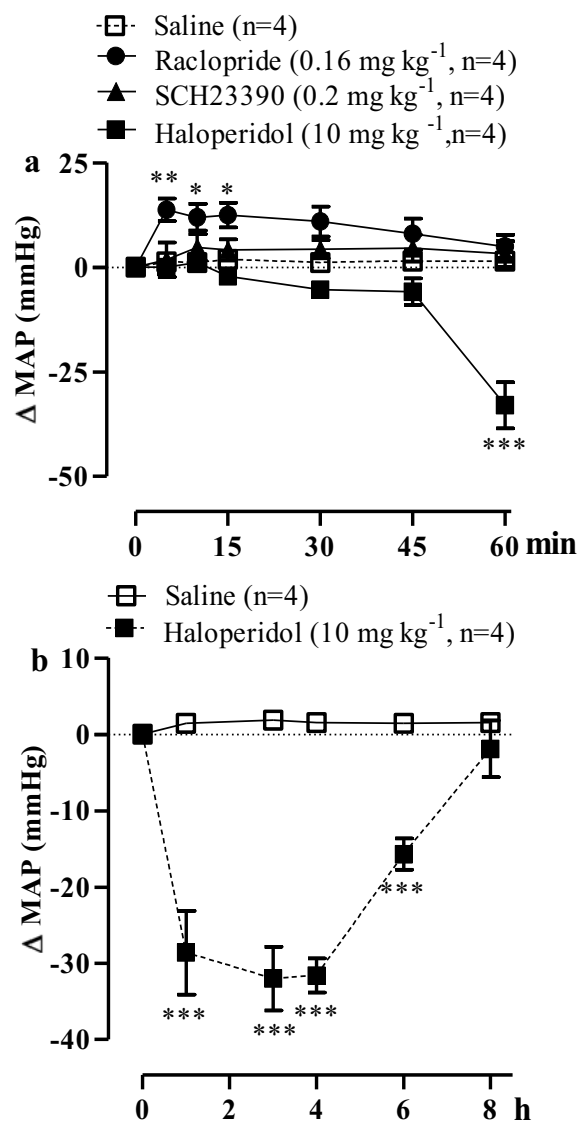
De Brito Gariepy et al.
Figure 6



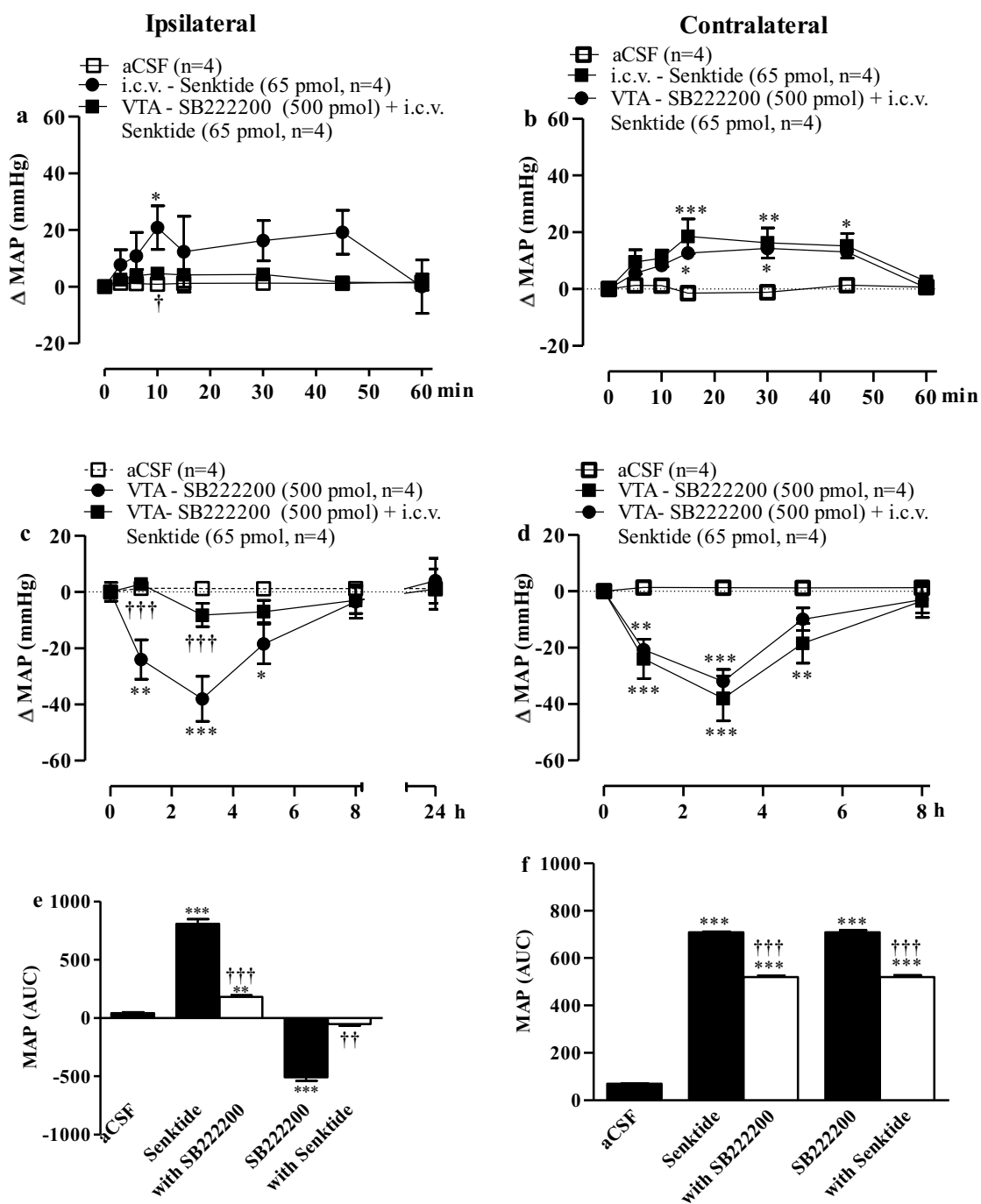
De Brito Gariepy et al.
Figure 7



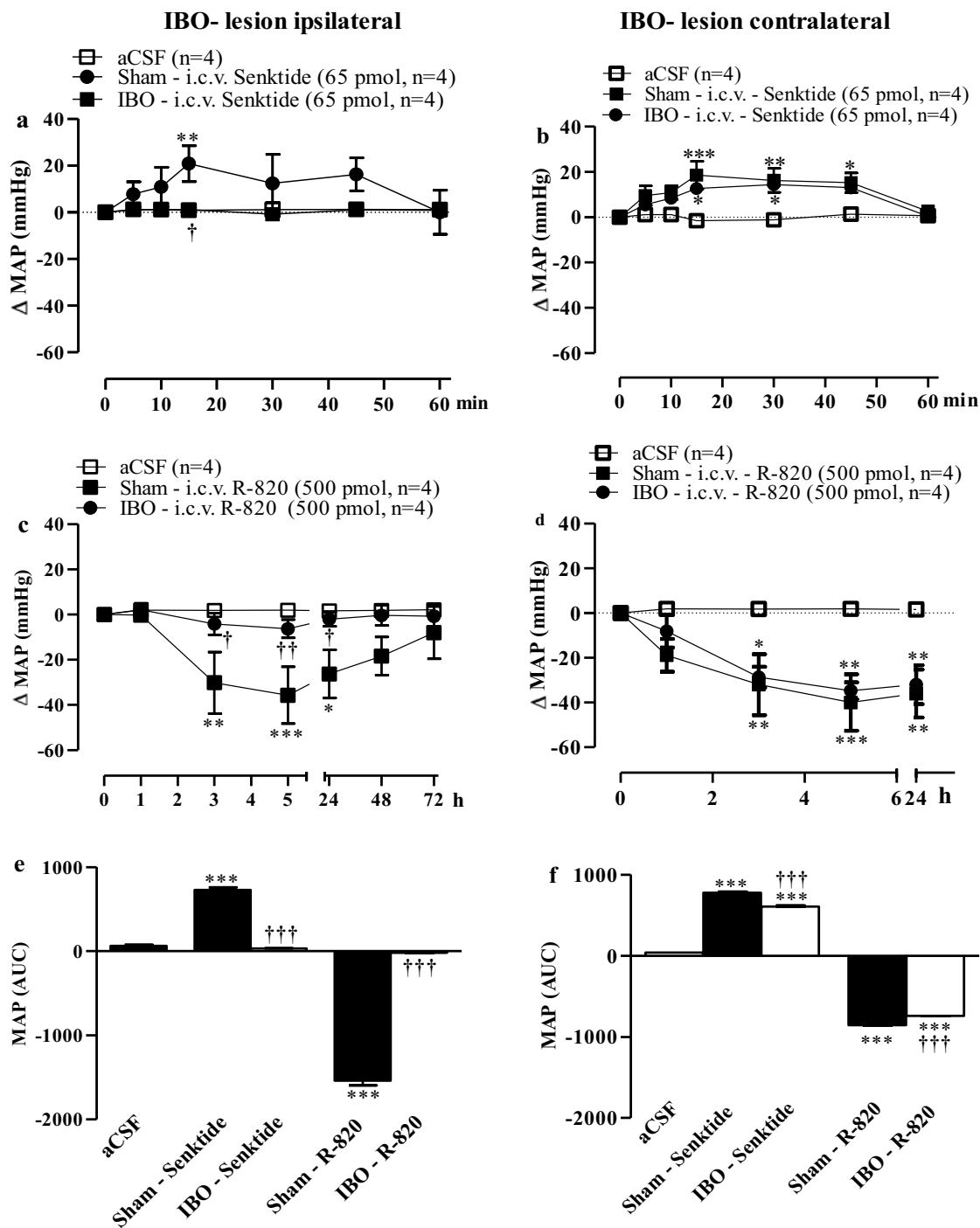
De Brito Gariépy et al.
Figure 8



De Brito Gariepy et al.
Figure 9



De Brito Gariepy et al.
Figure 10



De Brito Gariepy et al.
Figure 11

12.2 Article #2

Contributions:

H. De Brito Garipey realized the experiments and collaborated in the writing of the manuscript. P. Carayon and B. Ferrari provided the SSR240612 compound. R. Couture contributed in the redaction and revision of the manuscript.

Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension

H. De Brito Gariepy ^a, P. Carayon ^b, B. Ferrari ^b, R. Couture ^{a,*}

^a Département de Physiologie, Faculté de médecine, Université de Montréal, C.P. 6128 Succursale Centre-ville, Montréal, QC, Canada - H3C 3J7

^b Sanofi-Aventis R&D, 371 Rue du Professeur Blayac, Montpellier, France - 34184

Article published in *Neuropeptides* Vol 44, Issue 2, pages 191 to 198, 2010.

*Corresponding author:

Tel.: +1 514 343 7060

Fax: +1 514 343 2111

Abstract

Kinins are neuroactive peptides that could play a role in central autonomic control of blood pressure. Whereas kinin B1R binding sites were increased in specific brain areas of spontaneously hypertensive rats (SHR) and Angiotensin II (AngII)-hypertensive rats, the contribution of kinin B1R in hypertension remains controversial. The aims of the study were to determine: a) the effects on mean arterial blood pressure (MAP) of centrally and peripherally administered B1R antagonists in SHR (16 weeks) and AngII-hypertensive rats (200 ng/kg/min \times 2 weeks, s.c.); b) the contribution of central dopamine in the effects of SSR240612. The rationale is based on the overactivity of the dopaminergic system in hypertension. In both models, SSR240612 (1, 5, 10 mg/kg, gavage) reduced dose-dependently MAP (\geq -75 mmHg at least up to 6-8 h) and this therapeutic effect was resolved after 24 h. At the dose of 5 mg/kg, SSR240612-induced anti-hypertension was prevented by two dopamine receptor blockers, namely raclopride (0.16 mg/kg i.v.) and haloperidol (10 mg/kg s.c.). I.c.v. SSR240612 (1 μ g) decreased rapidly MAP in both models (1-6h) via a raclopride sensitive mechanism. In comparison, peripherally acting B1R antagonists (R-715 and R-954, 2 mg/kg s.c.) caused shorter and very modest decreases of MAP (-20 to -30 mmHg). Centrally or peripherally administered B1R antagonists had no effect on MAP in control Wistar-Kyoto rats. Data provide the first pharmacological evidence that the upregulated brain kinin B1R contributes through a central dopaminergic mechanism (DA-D2R) to the maintenance of arterial hypertension in genetic and experimental animal models of hypertension.

Keywords: Kinin B1R antagonist, blood pressure, dopamine, cardiovascular effect.

1. Introduction

Kinins which refer to bradykinin (BK) and kallidin (Lys-BK) are vasoactive peptides regulating the vasomotor tone and putative mediators involved in the central autonomic control of blood pressure (BP) (Couture and Lindsey, 2000). These 9-10 amino acid peptides exert their effect through the activation of two transmembrane G-protein-coupled receptors denoted as B1 and B2. The genes encoding the two receptors (R) have been cloned in several animal species and the organisation of the mammalian B1B2 receptor gene locus was identified (Cayla et al., 2002). The B2R is constitutive and mediates most of the biological effects of BK and kallidin. However, the B1R has a low level of expression in normal physiological conditions, yet it is readily expressed and induced following inflammation, tissue injury or in conditions of hyperglycemia/diabetes associated with the oxidative stress (Couture and Girolami, 2004; Lungu et al., 2007; Ismael et al., 2008). The preferential agonists of the B1R are the C-terminal fragments des-Arg⁹-BK and Lys-des-Arg⁹-BK (Regoli et al., 2001). This receptor is a potential pharmacological target for chronic inflammation and various cardiovascular and neurological diseases (Bader et al., 2000; Rodi et al., 2005; Leeb-Lundberg et al., 2005).

Recently, we reported that two non-peptide kinin B1R antagonists (LF22-0542, SSR240612) reversed dose- and time-dependently high systolic BP when given acutely while SSR240612 caused a persistent normalization of high BP when given chronically to glucose-fed rats, a model of insulin resistance (Lungu et al., 2007; Dias et al., 2009). The anti-hypertensive effect of these antagonists was most likely centrally mediated because R-715 (AcLys [D-βNal⁷, Ile⁸] des-Arg⁹-BK), a peptide B1R antagonist, which does not pass the blood-brain barrier, did not alter BP (Lungu et al., 2007). Nevertheless, the role of B1R in hypertension remains controversial.

Martins et al. (1991) reported that neither the B1R agonist des-Arg⁹-BK nor the B1R antagonist Leu⁸-des-Arg⁹-BK injected into the fourth ventricle altered BP in spontaneously hypertensive rat (SHR) or Wistar-Kyoto rat (WKY). In contrast, Alvarez et al. (1992) reported that the same antagonist injected into the lateral ventricle (i.c.v.) caused a long lasting reduction in BP and heart rate (HR) in SHR but not in WKY. Emanuelli et al. (1999) reported that i.c.v. activation of B1R in SHR and WKY evokes increases in BP, while i.c.v. injection of R-715 causes a mild decrease of BP (-14 mmHg) within 15 min in SHR. The latest findings were, however, not reproduced with the same peptide B1R agonist and antagonist in SHR (Cloutier et al., 2004).

Studies by autoradiography showed elevated densities of B1R binding sites in cortical areas, thalamic regions, amygdala and hippocampus, and in some pressor-related hypothalamic areas of 16-week-old SHR, such as dorsal hypothalamic area and ventromedial hypothalamic nucleus (de Wardener, 2001; Cloutier et al., 2004). Densities of B1R binding sites were very low in all examined brain structures in young SHR, and only small differences were found between SHR and WKY at the age of 8 weeks (Cloutier et al., 2004). This is consistent with increased B1R mRNA expression in the hypothalamus of 12- to 13-week-old SHR (Qadri et al., 2002).

In the model of hypertension induced by a 2-week treatment with Angiotensin II (AngII), B1R binding sites were markedly increased in the spinal cord (Petcu et al., 2005) and in some specific brain regions (SN, septum, posterodorsal tegmental nucleus and temporal cortex) (De Brito Pereira et al., 2008). The inhibition of the oxidative stress with a diet supplemented with alpha-lipoic acid reversed the up-regulation of B1R, arterial hypertension and the increased production of vascular superoxide anion, suggesting that B1R is induced by the oxidative stress and contributes to hypertension in this model (Petcu et al., 2005).

The present study was undertaken to determine whether or not the anti-hypertensive effect of the B1R antagonist SSR240612 seen in glucose-fed rats could also be reproduced in SHR and AngII-treated rats, two classical models of hypertension. In order to determine whether the anti-hypertension is centrally or peripherally mediated, BP effects of SSR240612 administered by gavage and i.c.v. were compared, and two peptide antagonists (R-715 and R-954), which do not pass the blood brain barrier (Regoli et al., 2001), were tested. It was recently found that brain dopamine D2 receptor (DA-D2R) is involved in the anti-hypertensive effect of tachykinin NK-3R antagonists in SHR and AngII-hypertensive rats (De Brito Pereira and Couture 2007). This is congruent with the increased central dopaminergic activity reported in SHR (Amenta et al., 2001). Hence, the contribution of central DA in the anti-hypertensive effect of SSR240612 was examined as a putative mechanism.

2. Materials and methods

2.1. *Animal source and care*

Male SHR (15-16 weeks) and age-matched WKY (200-225g) were purchased one week prior to experiments from Charles River (St Constant, Québec, Canada). They were housed two per cage under a 12 h light-dark cycle in a room with controlled temperature (22°C) and humidity (40%) with food (Charles River Rodent) and tap water available *ad libitum*. The care of animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Animal Care Committee of our University.

2.2. *Surgery*

Under isoflurane anesthesia, WKY was implanted subcutaneously with an osmotic mini pump (Alzet 2002, Alza Corporation, Palo Alto, CA, USA) to deliver AngII (200 ng/kg/min) for 14 days as described previously

(Laplane et al., 2003). Control rats were sham-operated WKY having no implanted mini pump. Ten days later, rats were anesthetized with isoflurane and an i.c.v. guide cannula was implanted into the left lateral brain ventricle as described earlier (Lessard et al., 2004). Four days later, rats were reanesthetised and a catheter (PE-10 connected to PE-60) was inserting into the abdominal aorta through the femoral artery for direct BP recording and exteriorized at the back of the neck. Under similar procedures, i.c.v. and vascular catheters were implanted in SHR and control WKY. Before each surgery, the animals received the antibiotics trimethoprim and sulphadiazine (Tribissen 24%, 30 mg/kg, s.c., Schering Canada Inc., Pointe Claire, Québec, Canada) and an analgesic drug (Anafen, 10 mg/kg, s.c., Merial Canada Inc., Baie d'Urfé, Québec, Canada). Thereafter, the rats were allowed to recover in individual plastic cages (40 cm X 23 cm X 20 cm) and housed in the same controlled conditions. Experimental protocols were initiated 24 after the intravascular surgery, in freely behaving rats.

2.3. Measurement of blood pressure and i.c.v. injections

Rats were transported to an isolated and quiet testing room where only the experimenter had access. They remained in their resident cage and had free access to food and water. One hour after their transportation to the testing room, BP was recorded on a high-performance data acquisition system PowerLab 8/30 with LabChart Pro ML870P and ML228 Bridge Amp (ADInstruments Inc, Colorado Springs, CO, USA). Mean arterial blood pressure (MAP) was calculated from systolic (S) and diastolic (D) blood pressure values $((S - D)/3 + D = \text{MAP})$.

I.c.v. injections were made when BP was stable; a 31 G stainless-steel injector connected to a Standard Syringe 5 μl (Fisher Scientific Ltd, Montréal, Québec, Canada) with PE-10 tubing was inserted into the cannula-guide without handling the rat. The injector was removed from the guide cannula 1 min after injection to prevent any possible leakage of

the injectate (Cloutier et al., 2004). The correct position of the i.c.v. catheter was verified by post-mortem examination at the end of experiment.

2.4. Experimental protocols

2.4.1. Experiment 1: effect of orally administered SSR240612

AngII-treated rats and SHR received by gavage the B1R antagonist SSR240612 (1, 5 and 10 mg/kg) and BP was measured for up to 6-8 h. Rats were reconnected 24 h later to see if BP was back to baseline values. They received first a single dose of 5 mg/kg and 24-48h later, the dose of 10 mg/kg. The dose of 1 mg/kg was given to a separate group of AngII-treated rats and SHR. Comparison was made to vehicle values (similar volume of SSR240612 vehicle given by gavage) and to control WKY that received by gavage 5 mg/kg SSR240612.

2.4.2. Experiment 2: effects of SSR240612 in the presence of dopamine receptor antagonists

Groups of AngII-treated rats and SHR received by gavage SSR240612 (5 mg/kg) and the effect on BP was recorded continuously for a period of 8h. The reversibility of the B1R antagonist effect on BP was assessed 24h later. Then rats received an i.v. injection of the DA-D2R antagonist raclopride (0.16mg/kg), 30 min prior to assessing again the effect of SSR240612 (5 mg/kg) on BP. Raclopride was injected alone 24 h later to assess its direct effect on BP. Control WKY rats received only raclopride at the same dose. Groups of AngII-treated rats and SHR received by gavage SSR240612 (5 mg/kg) and the effect on BP was measured continuously for a period of 6-8h. The reversibility of the B1R antagonist effect on BP was assessed 24h later. Then rats were treated subcutaneously (s.c.) with the dopamine antagonist haloperidol (10 mg/kg) 30 min prior to assessing again the effect of SSR240612 (5 mg/kg) on BP. On the subsequent day, SSR240612 (5 mg/kg) was re-administered to confirm the reversibility of any blockade observed in the presence of haloperidol on the previous day. The dopamine antagonist haloperidol (10 mg/kg) was injected alone 24 h later

to assess its direct cardiovascular effect. The same treatment with haloperidol was given to control WKY.

2.4.3. Experiment 3: effect of i.c.v. administered SSR240612

I.c.v. injections in AngII-treated rats, SHR and control WKY were initiated with an injection of aCSF; this was followed 60 min later by a single dose of kinin B1R antagonist SSR240612 (1µg/µl) followed by 4 µl volume of aCSF, which corresponds to the void volume of the catheter. BP was measured continuously for a period of 6h post-injection. The following day, rats received an i.v. injection of the DA-D2R antagonist raclopride (0.16mg/kg), 30 min prior to assessing the effect of SSR240612 (5 mg/kg) on BP. Baseline BP was recorded 24h later. Rats which received i.c.v. injection did not receive SSR240612 by gavage.

2.4.4. Experiment 4: peripheral effects of R-715 and R-954

AngII-treated rats, SHR and their control WKY received the peptide B1R antagonist R-954 (2 mg/kg, s.c.) and the effect on BP was measured for up to 10-12 h post-injection. On the following day, the same rats received R-715 (2 mg/kg, s.c.) and the BP was recorded for up to 10-12 h post-injection.

2.5. Drugs and solutions

aCSF was purchased from Harvard Bioscience (Massachusetts, U.S.A.) and Angiotensin II from Sigma-Aldrich Canada. SSR240612 [(2*R*)-2-[(3*R*)-3-(1,3-benzodioxol-5-yl)-3-[(6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl]amino]-3-(4-{[2*R*,6*S*]-2,6-dimethylpiperidinyl)methyl}phenyl)-*N*-isopropyl-*N*-methylpropanamide hydrochloride] (Gougat et al., 2004) was synthesized by Sanofi-Aventis (Montpellier, France). It was dissolved in dimethylsulphoxide (0.5%), and then ethanol (5%) and Tween-80 (5%) were added in this sequence and completed with distilled water (30mg/kg) (Dias et al., 2007). R-715

(AcLys[D- β Nal⁷,Ile⁸] des-Arg⁹-BK and R-954 (Ac-Orn-[Oic², α -MePhe⁵, D- β Nal⁷, Ile⁸]-desArg⁹-BK) were provided by Dr. Fernand Gobeil (Department of Pharmacology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada) and dissolved in saline.

Raclopride (selective DA-D2R antagonist) used at 0.16 mg/kg (Millan et al., 1998) and haloperidol (non selective DA-D2R antagonist) used at 10mg/kg (Backhouse et al., 1982) were purchased from Sigma-Aldrich Canada. Raclopride was dissolved in DMSO and completed in saline (final solution contains 3% DMSO) while haloperidol was dissolved in ethanol and DMSO and completed in saline (final solution contains 3% ethanol and 0.5% DMSO). Haloperidol has high affinity for the DA-D2R (Miyamoto et al., 2005) and for sigma 1 and sigma 2 receptors (Hashimoto and Ishiwata 2006) whereas it shows lower affinity for DA-D1, D3 or D4 receptors, serotonin 2A receptor, and α 1 adrenergic receptor (Miyamoto et al., 2005).

2.6. Statistical analysis of data

Results are expressed as means \pm s.e.m. of values obtained from (*n*) rats. Statistical analysis of data was performed with Graph-Pad Prism software. Time course effects were analyzed for statistical significance by a two-way analysis of variance (ANOVA) followed by a Bonferroni test for multiple comparisons. A one-way ANOVA followed by a *post hoc* Dunnett's test was used for multiple comparisons to the same control group (maximal values and area under the curve (AUC). Only probability values (*P*) less than 0.05 was considered to be statistically significant.

3. Results

3.1. Anti-hypertensive effect caused by SSR240612 in AngII-treated rats

AngII-induced hypertension (MAP = 187.9 ± 6.4 mmHg, $n=17$) was significantly reversed to hypotensive values (77.2 ± 4.8 mmHg) by the kinin B1R antagonist SSR240612 (5 and 10 mg/kg) at 3 and 6 h post-gavage. The response was resolved at 24 h. When the AUC was taken into account, the dose of 1mg/kg SSR240612 produced a small but significant depressor effect when compared to vehicle (Fig. 1a, c). In contrast, SSR240612 (5mg/kg) failed to cause significant changes in MAP in control WKY.

3.2. Anti-hypertensive effect caused by SSR240612 in SHR

SSR240612 (1, 5 and 10 mg/kg) dose-dependently decreased MAP between 1 and 6h post-gavage in SHR when compared to vehicle (Fig 2a,c). High MAP in SHR (187.2 ± 4.8 mmHg, $n = 5$) was reduced by 1 mg/kg (167.2 ± 4.6 mmHg) at 3h and normalized by 5 mg/kg (115.7 ± 7.6 mmHg) between 3 and 6h post-gavage.

3.3. Blockade of the anti-hypertensive effect caused by SSR240612 in both animal models

The anti-hypertensive effect of SSR240612 (5mg/kg) from 1 to 8h post-gavage was significantly blocked by the DA-D2R antagonist raclopride (0.16mg/kg, i.v.) administered 30 min earlier in both AngII-treated rats (Fig. 1b,d) and SHR (Fig. 2b,d). Whereas raclopride had no significant effect on MAP in AngII-treated rats and control WKY (Fig. 1b, d), the DA-D2R antagonist enhanced significantly MAP in SHR (Fig. 2b, d) during the recording period of 6h.

The anti-hypertensive response to SSR240612 (5 mg/kg) was significantly reduced by haloperidol (10 mg/kg, s.c.) administered 30 min earlier in AngII-treated rats (Fig. 3 a, c) and SHR (Fig. 3b, d). When SSR240612 was re-administered alone 24h after haloperidol treatment, the anti-hypertensive effect of SSR240612 was similar to that measured on the first day in the absence of haloperidol (data not shown). Haloperidol (10

mg/kg, s.c.) had a direct inhibitory effect on MAP in both AngII-treated rats and SHR (Fig. 3). However, the same treatment with haloperidol in control WKY had no significant effect on MAP.

3.4. Anti-hypertensive effect induced by i.c.v. injection of SSR240612

When SSR240612 was injected directly into the left cerebral ventricle at a dose of 1 µg, it caused a significant reduction of MAP in both AngII-treated rats and SHR (Fig. 4). The effect was significant at 1 h post-injection and lasted during the whole recording period of 6 h; all rats had returned to their hypertensive state 24h later. The anti-hypertensive response induced by i.c.v. SSR240612 was significantly reduced by raclopride (0.16 mg/kg, i.v.) given 30 min earlier in both models of hypertension (Fig. 4).

3.5. Changes of MAP induced by R-715 and R-954

In AngII-treated rats (Fig. 5a, c) and SHR (Fig. 5 b, d) both B1R antagonists R-954 and R-715 (2mg/kg, s.c.) evoked a modest (-20 to -30 mmHg) but significant decrease in MAP when AUC values were compared to vehicle. This effect was seen between 1 and 6-8h post-injection. The same treatments with R-954 and R-715 in control WKY had no significant effect on MAP.

4. Discussion

The present findings show that SSR240612 caused a pronounced anti-hypertensive effect in SHR and AngII-treated rats through a central mechanism involving DA-D2R. This hypothesis is supported by the following observations: 1- the blockade of peripheral B1R with R-954 and R-715 caused a less striking decrease of MAP; 2- the anti-hypertensive effect of SSR240612 is reproduced when injected directly in the brain ventricles; 3- both the systemic and i.c.v. anti-hypertensive effects of SSR240612 are prevented by raclopride or haloperidol. Hence, our data suggest that B1R may contribute to the overactivity of the DA system reported in SHR (van den Buuse, 1997). This conclusion can be extended to

the model of AngII-induced hypertension in which raclopride and haloperidol reversed the anti-hypertensive effect of SSR240612. Therefore, the anti-hypertensive effect of B1R antagonists is not restricted to the model of insulin resistance induced by high glucose feeding (Lungu et al., 2007; Dias et al., 2009) and can be generalised to classical models of hypertension.

SSR240612 is a highly potent, selective non-peptide antagonist of the B1R in all species tested (rat, mouse, rabbit and human) and is orally active. The selectivity for B1R versus B2R was in the range of 500- to 1000-fold and was found inactive when tested over 100 receptor-binding, ion channel-binding, and enzyme assays including dopamine DA-D1R and DA-D2R (Gougat et al., 2004). Therefore the anti-hypertensive effect of SSR240612 in SHR and AngII-treated rats is unlikely a direct interaction with central DA receptors. SSR240612 was inactive on blood pressure in normotensive control WKY rats which do not express a significant level of B1R. This is a strong indication that SSR240612 interacts only with B1R when sufficiently expressed as in the present models of hypertension. Since the anti-hypertensive effect of SSR240612 is lost when DA-D2R is blocked with raclopride or haloperidol, it is reasonable to suggest that SSR240612 reverses hypertension by reducing the overactivation of central DA-D2R mediated by B1R. The tonic activation of DA neurons through B1R can be direct or indirect through the release of other central neuromediators. Electrophysiological studies in addition to immunocytochemical co-localization of B1R on DA neurons (terminals, dendrites and/or perikarya) are compelling to define the exact mechanism by which SSR240612 acts as anti-hypertensive drug.

Although B1R is up-regulated in specific brain areas of SHR and AngII-induced hypertension (see Introduction), the possibility that endogenous B1R ligands are increased in hypertension is still unknown. Nevertheless, higher levels of kallikrein activity were detected in the cerebrospinal fluid of SHR and desoxycorticosterone-acetate-salt hypertensive rats in comparison with their normotensive controls (Khan et al., 1993; 1995).

Similarly, kininogen was found significantly increased in CSF of SHR (Alvarez et al., 1992). In young and adult SHR, most studies showed that kinin concentrations in CSF and in specific areas (hypothalamus and septum) are significantly larger than those of age-matched normotensive WKY (reviewed by Couture and Lindsey, 2000). The expression of B2R is also significantly enhanced in the brain and spinal cord of SHR and contributes to the hypersensitivity of the cardiovascular response to BK (Cloutier et al., 2002; Ongali et al., 2003; Cloutier et al., 2004). Taken together, data suggest that the activity of the central kallikrein-kinin system is increased in hypertension.

To investigate the importance of kinin B1R in central regulation of BP, we used a pharmacological approach. Discrepancy between earlier studies regarding the central anti-hypertensive effect of B1R antagonists in SHR (see Introduction) can be related to various factors and experimental conditions, including the recording period of BP and the physico-chemical features of the antagonists. For instance, the lipophilic non-peptide SSR240612 is expected to have a greater brain tissue diffusion and distribution than the previously used peptide antagonists (Leu⁸-des-Arg⁹-BK and R-715) whose effect might be limited to B1R located around the circumventricular regions upon i.c.v. administration. The reduction of BP induced by i.c.v. SSR240612 displayed a slow onset and occurred after 30 min post-injection and was no longer seen when rats were recorded again 24h later. Thus the 1 to 6h post-injection window might have been missed in our earlier study using R-715 as we expected to see an acute effect within 30 min on baseline BP (Cloutier et al., 2004). Indeed, the mild anti-hypertensive effect reported earlier with R-715 by Emanuelli et al., (1999) was seen within the first minute post-i.c.v. injection and was maximal at 15 min.

Central DA activity is altered in SHR in comparison with WKY and the role of central DA in the development of hypertension has been highlighted

(van den Buuse, 1991; 1997). An up-regulation of dopamine D1R and D2R in the caudate-putamen and nucleus accumbens, one major neuronal projection of the ventral tegmental area, was reported in young SHR (Kirouac and Ganguly, 1993; Vaughan et al., 1999). Furthermore, D2R mRNA expression was found enhanced in the SN of young and old SHR (Vaughan et al., 1999), a region of the nigrostriatal system where B1R binding sites were significantly increased in comparison with WKY (De Brito Pereira et al., 2008). Lesion of the nigrostriatal DA pathway attenuates the development of hypertension in young SHR (van den Buuse et al., 1991; Linthorst et al., 1994) and electrical or chemical stimulation of *pars compacta* of SN leads to increases of heart rate, BP and striatal DA levels in anesthetized rats (Lin and Yang, 1994). Whereas activation of the brain pre-synaptic DA-D2R decreased BP, stimulation of post-synaptic DA-D2R increased BP (Jose et al., 1999). Thus the mesolimbic and nigrostriatal dopaminergic systems may represent strategic sites for the anti-hypertensive effects of B1R antagonist. This would be reminiscent of the role played by tachykinins on these systems in the autonomic control of BP and in the maintenance of hypertension in SHR (Lessard et al., 2003; Deschamps and Couture, 2005).

Whereas the selective blockade of DA-D2R with raclopride produced a slight vasopressor response that reached significance only in SHR, haloperidol was effective in reversing high blood pressure in SHR and AngII-treated rats. The non-selectivity of haloperidol versus raclopride for DA receptors is likely to exert a more complete blockade of DA action which can account for its pronounced anti-hypertensive response. This supports the overactivity of the DA system in hypertension as both DA antagonists were inactive in normotensive control WKY.

In conclusion, data provide the first pharmacological evidence that the upregulated cerebral kinin B1R contributes through a central dopaminergic mechanism (DA-D2R) to the maintenance of arterial hypertension in

genetic and experimental animal models of hypertension. The findings with R-715 and R-954 which unlikely cross the blood-brain barrier point to a small contribution of peripheral B1R in hypertension. Hence, centrally active non-peptide B1R antagonists with oral bioavailability could be of therapeutic value in the treatment of arterial hypertension.

Conflict of interest statement

Drs P. Carayon and R. Couture hereby declare a duality of interest in view of their holding a patent made available to public in 2008 for the use of a non-peptide kinin B1 receptor antagonist in the treatment of hypertension (Publication No 2916352).

Acknowledgements

This work was supported by the Canadian Institutes of Health Research (CIHR) (MOP-79471).

References

- Alvarez, A.L., Delorenzi, A., Santajuliana, D., Finkielman, S., Nahmod, V.E., Pirola, C.J., 1992. Central bradykininergic system in normotensive and hypertensive rats. *Clin. Sci.* **82**, 513-519.
- Amenta F., Ricci, A., Rossodivita, I., Avola, R., Tayebati, S.K., 2001. The Dopaminergic System in Hypertension. *Clin. and Exper. Hypertension* **23**, 15-24.
- Bader, M., Bohnemeier, H., Zollmann, F.S., Lockley-Jones, O.E., Ganten, D., 2000. Transgenic animals in cardiovascular disease research. *Exp. Physiol.* **85**, 713-731.
- Backhouse, B., Barochovsky, O., Malik, C., Patel, A.J., Lewis, P.D., 1982. Effects of haloperidol on cell proliferation in the early postnatal rat brain. *Neuropathol. Appl. Neurobiol.* **8**, 109-116.
- Cayla, C., Merino, V.F., Cabrini, D.A., Silva, J.A., Jr., Pesquero, J.B., Bader, M., 2002. Structure of the mammalian kinin receptor gene locus. *Int. Immunopharmacol.* **2**, 1721-1727.
- Cloutier, F., de Sousa Buck, H., Ongali, B., Couture, R., 2002. Pharmacologic and autoradiographic evidence for an up-regulation of kinin B(2) receptors in the spinal cord of spontaneously hypertensive rats. *Br. J. Pharmacol.* **135**, 1641-1654.
- Cloutier, F., Ongali, B., Campos, M.M., Thibault, G., Neugebauer, W., Couture, R. 2004. Correlation between brain bradykinin receptor binding sites and cardiovascular function in young and adult spontaneously hypertensive rats. *Br. J. Pharmacol.* **142**, 285-296.
- Couture, R., Lindsey, C.J., 2000. Brain kallikrein-kinin system: from receptors to neuronal pathways and physiological functions. *Hand. Chem.*

Neuroanat. Vol. 16: Peptide Receptors Part 1, Eds: R. Quirion, A. Björklund, T. Hökfelt, Elsevier Science B.V., 241-300.

Couture, R., Girolami, J.P., 2004. Putative roles of kinin receptors in the therapeutic effects of angiotensin 1-converting enzyme inhibitors in diabetes mellitus. *Eur. J. Pharmacol.* 500, 467-485.

De Brito Pereira, H., Couture, R., 2007. Brain tachykinin NK-3 receptors contribute to arterial hypertension through a dopaminergic mechanism. 38th Annual Meeting of Society for Neuroscience, San Diego, CA, USA, Nov. 3-7, 2007, (P296.12).

De Brito Pereira, H. Talbot S., Ongali B., Couture R., 2008. Role of kinin B1 receptor in the central autonomic control of blood pressure and body temperature in two rat models of hypertension. 39th Annual Meeting of the Society for Neuroscience, Washington, DC, USA, Nov. 12-15, 2008 (P83.18).

Deschamps K., Couture, R., 2005. The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br. J. Pharmacol.* **145**, 712-727.

De Wardener, H.E., 2001. The Hypothalamus and Hypertension. *Physiol. Rev.* 81, 1599-1658.

Dias, J.P., Talbot, S., Pilon, M., Sénécal, J., de Champlain, J., Ferrari, B., Carayon, P., Couture, R., 2009. Kinin B1 receptor as a therapeutic target in the treatment of diabetes. The 5th International Symposium on Peptide Receptors and Kinin 2009, June 26-30, Quebec, Canada (P28).

Dias, J.P., Ismael, M.A., Pilon, M., de Champlain, J, Ferrari, B., Carayon, P., Couture, R., 2007. The kinin B1 receptor antagonist SSR240612 reverses tactile and cold allodynia in an experimental rat model of insulin resistance. *Br. J. Pharmacol.* 152, 280-287.

Emanuelli, C., Chao, J., Regoli, D., Chao, L., Ni, A., Madeddu, P., 1999. The bradykinin B₁ receptor and the central regulation of blood pressure in spontaneously hypertensive rats. *Br. J. Pharmacol.* **126**, 1769-1776.

Gougat, J., Ferrari, B., Sarran, L., Planchenault, C., Poncelet, M., Maruani, J., Alonso, R., Cudennec, A., Croci, T., Guagnini, F., Urban-Szabo, K., Martinolle, JP., Soubrié, P., Finance, O., Le Fur, G. J., 2004. SSR240612[(2R)-2-[(3R)-3-(1,3-benzodioxol-5-yl)-3-[(6-methoxy-2-naphthyl) sulfonyl] amino] propanoyl) amino]-3-(4-[[2R,6S)-2,6-dimethylpiperidiny]methyl]phenyl)- N-isopropyl-N-methylpropanamide hydrochloride], a new nonpeptide antagonist of the bradykinin B₁ receptor: biochemical and pharmacological characterization. *J. Pharmacol. Exp. Therap.* 309, 661-669.

Hashimoto, K., Ishiwata, K., 2006. Sigma receptor ligands: possible application as therapeutic drugs and as radiopharmaceuticals. *Curr Pharm Des.* 12, 3857-3876.

Ismael, M.A., Talbot, S., Carbonneau, C.L., Beauséjour, C.M., Couture, R., 2008. Blockade of sensory abnormalities and kinin B₁ receptor expression by N-Acetyl-L-Cysteine and ramipril in a rat model of insulin resistance. *Eur. J. Pharmacol.* 589, 66-72.

Jose, P.A., Eisner, G.M., Felder, R.A., 1999. Role of dopamine in the pathogenesis of hypertension. *Clin. Exp. Pharmacol. Physiol. Suppl.* 26, 10-13.

Khan, I.M., Yamaji, I., Miller, D.H., Margolius, H.S., Privitera, P.J., 1993. Cerebrospinal fluid kallikrein in spontaneously hypertensive and desoxycorticosterone acetate-salt hypertensive rats. *J. Hypertens.* 11, 1039-1045.

Khan, I.M., Miller, D.H., Strickland, J., Margolius, H.S., Privitera, P.J., 1995. Brain kallikrein-kinin system abnormalities in spontaneously hypertensive rats. *Hypertension* 25, 524-530.

Kirouac, G.J., Ganguly, P.K., 1993. Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: an autoradiographic analysis. *Neuroscience* 52, 135-41.

Laplace, M.A., Wu, R., El Midaoui, A., de Champlain, J., 2003. NAD(P)H oxidase activation by angiotensin II is dependent on p42/44 ERK-MAPK pathway activation in rat's vascular smooth muscle cells. *J. Hypertens.* 21, 927-936.

Leeb-Lundberg, L.M., Marceau, F., Müller-Esterl, W., Pettibone, D.J., Zuraw, B.L., 2005. International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol. Rev.* 57, 27-77.

Lessard, A., Campos, M.M., Neugebauer, W., Couture, R., 2003. Implication of nigral tachykinin NK-3Rs in the maintenance of hypertension in spontaneously hypertensive rats: a pharmacologic and autoradiographic study. *Br. J. Pharmacol.* 138, 554-556.

Lessard, A., Laurin, M., Yamaguchi, N., Couture, R., 2004. Central anti-hypertensive effect of tachykinin NK-3R antagonists in rat. *Eur. J. Pharmacol.* 486, 75-83.

Lin, M.T., and Yang, J.J., 1994. Stimulation of the nigrostriatal dopamine system produces hypertension and tachycardia in rats. *Am. J. Physiol.* 266, H2489-2496.

Linthorst, A.C., van Giersbergen, P.L., Gras, M., Versteeg, D.H., de Jong, W., 1994. The nigrostriatal dopamine system: role in the development of hypertension in spontaneously hypertensive rats. *Brain Res.* 639, 261-268.

Lungu, C., Dias, J.P., França, C.E., Ongali, B., Regoli, D., Moldovan, F., Couture, R., 2007. Involvement of kinin B1 receptor and oxidative stress in sensory abnormalities and arterial hypertension in an experimental rat model of insulin resistance. *Neuropeptides* 41, 375-387.

Martins, D.T.O., Fior, D.R., Nakaie, C.R., Lindsey, C.J., 1991. Kinin receptors of the central nervous system of spontaneously hypertensive rats related to the pressor response to bradykinin. *Br. J. Pharmacol.* **103**, 1851-1856.

Millan, M.J., Newman-Tancredi, A., Brocco, M., Gobert, A., Lejeune, F., Audinot, V., Rivet, J.M., Schreiber, R., Dekeyne, A., Spedding, M., Nicolas, J.P., Peglion, J.L., 1998. S18126 ([2-[4-(2, 3-dihydrobenzo[1, 4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D₄ receptors: an *in vitro* and *in vivo* comparison with L 745, 870 (3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. *J. Pharmacol. Exp. Ther.* 287, 167-186.

Miyamoto, S., Duncan, G.E., Marx, C.E., Lieberman, J.A., 2005. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol. Psychiatry* 10, 79-104.

Ongali, B., Buck, H. de S., Cloutier, F., Legault, F., Regoli, D., Lambert, C., Thibault, G., Couture, R., 2003. Chronic effects of angiotensin-converting enzyme inhibition on kinin receptor binding sites in the rat spinal cord. *Am. J. Physiol. Heart Circ. Physiol.* 284, H1949-1958.

Petcu, M., Ongali, B., El Midaoui, A., de Champlain J., Couture, R., 2005. Effects of alpha-lipoic acid on kinin B1 and B2 receptor binding sites in the spinal cord of chronically angiotensin-treated rats. *Peptides*. 26, 1331-1338.

Qadri, F., Hauser, W., Jöhren, O., Dominiak, P., 2002. Kinin B1 and B2 receptor mRNA expression in the hypothalamus of spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.* **80**, 258-263.

Regoli, D., Rizzi, A., Perron, S.I., Gobeil, F. Jr., 2001. Classification of kinin receptors. *Biol Chem.* 382, 31-35.

Rodi, D., Couture, R., Ongali, B., Simonato, M., 2005. Targeting kinin receptors for the treatment of neurological diseases. *Curr. Pharm. Des.* 11, 1313-1326.

van den Buuse, M., Linthorst, A.C., Versteeg, D.H., de Jong, W., 1991. Role of brain dopamine systems in the development of hypertension in the spontaneously hypertensive rats. *Clin. Exp. Hypertens. A.* 13, 653-659.

van den Buuse, M., 1997. Pressor responses to brain dopaminergic stimulation. *Clin. Exp. Pharmacol. Physiol.* 24, 764-769.

Vaughan, C.E., van den Buuse, M., Roland, B.L., 1999. Brain dopamine D2 receptor mRNA levels are elevated in young spontaneously hypertensive rats. *Neurosci. Res.* 34, 199-205.

Figures Legends

Fig. 1: Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by three doses of SSR240612 (1, 5 and 10 mg/kg, gavage) in AngII-treated rats (a). Raclopride (0.16 mg/kg, i.v.) was injected alone or 30 min prior to 5 mg/kg SSR240612 to assess the participation of DA-D2R in the anti-hypertensive effect of SSR240612 (b). Control WKY also received 5 mg/kg SSR240612 (a) or 0.16 mg/kg raclopride (b). Area under the curves (AUC) was measured for a period of 0-6 h (c) or 0-8h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by ***P*<0.01; ****P*<0.001.

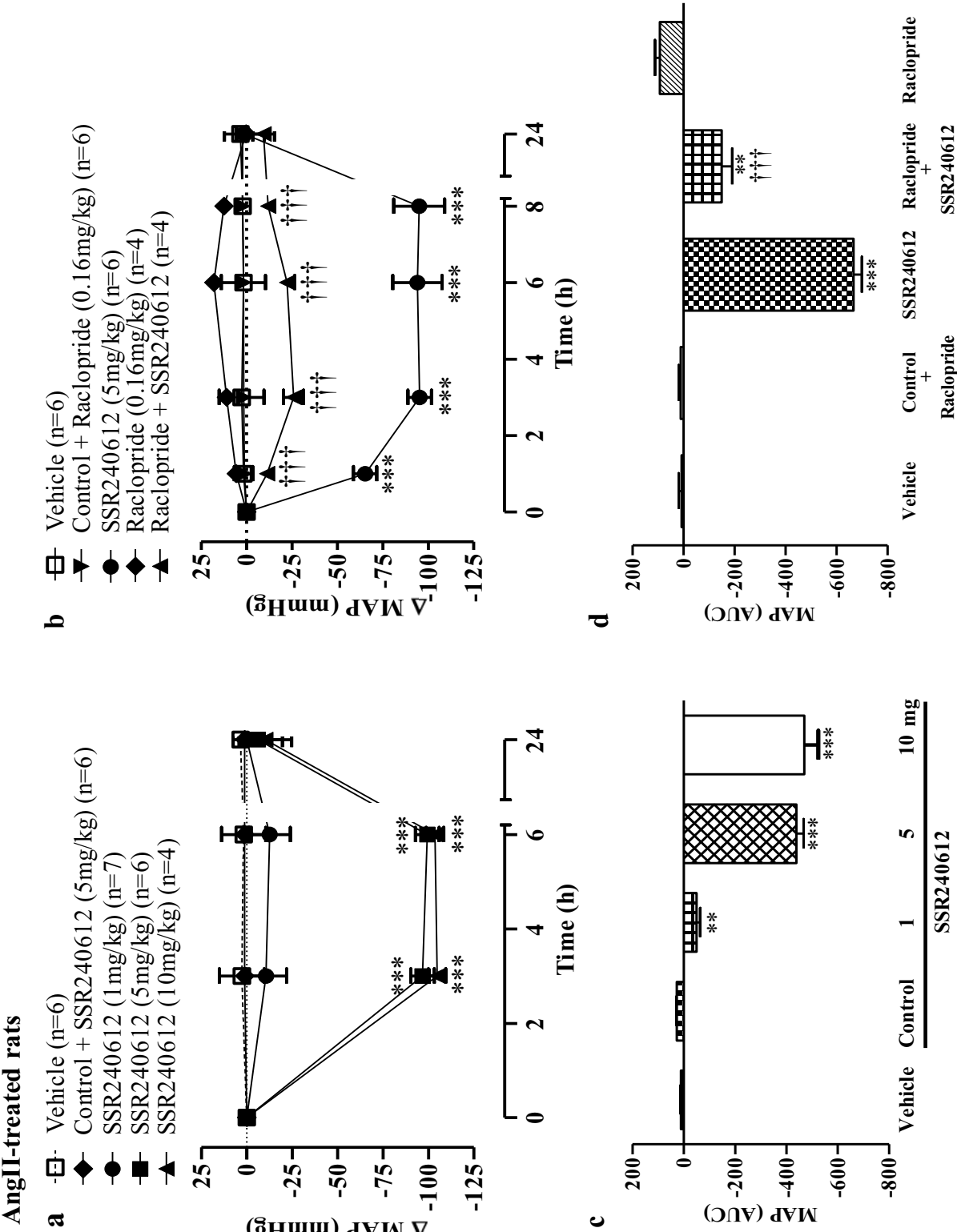
Fig. 2: Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by three doses of SSR240612 (1, 5 and 10 mg/kg, gavage) in SHR (a). Raclopride (0.16 mg/kg, i.v.) was injected alone or 30 min prior

to 5 mg/kg SSR240612 to assess the participation of DA-D2R in the anti-hypertensive effect of SSR240612 (b). Control WKY also received 5 mg/kg SSR240612 (see Fig 1a) or 0.16 mg/kg raclopride (b). Area under the curves (AUC) was measured for a period of 0-6 h (c,d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by **P*<0.05; ***P*<0.01; ***, ††*P*<0.001.

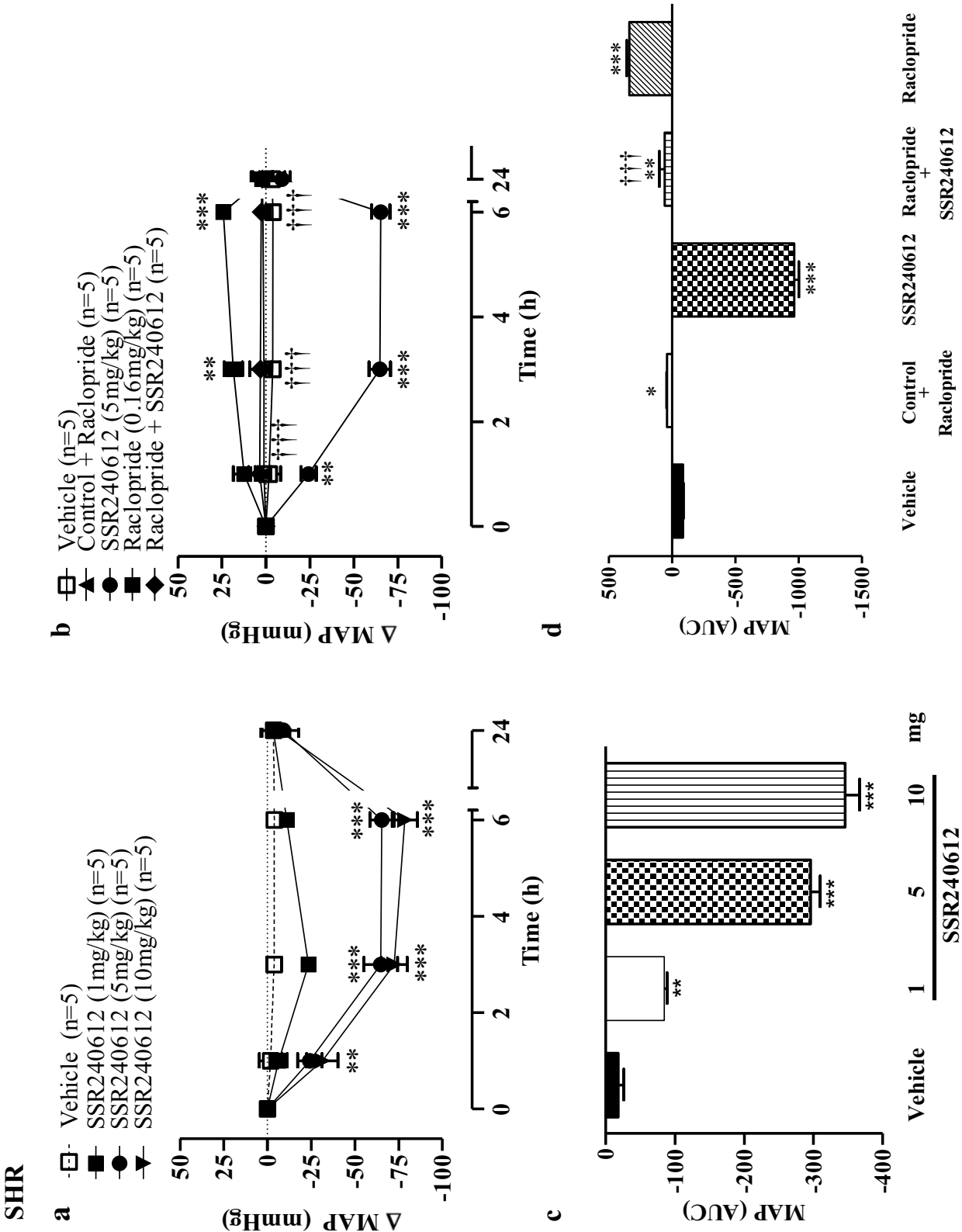
Fig. 3: Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by SSR240612 (5mg/kg, gavage) in AngII-treated rats (a) and SHR (b) before and 30 min after administration of the dopamine antagonist haloperidol (10 mg/kg, s.c). Hypertensive rats and control WKY also received 10 mg/kg haloperidol alone. Area under the curves (AUC) was measured for a period of 0-8 h (c) or 0-6 h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) or SSR240612 (†) is indicated by **P*<0.05; **, ††*P*<0.01; ***, †††*P*<0.001.

Fig. 4: Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by SSR240612 (1 μ g, i.c.v.) in AngII-treated rats (a) and SHR (b) before and 30 min after administration of the DA-D2R antagonist raclopride (0.16 mg/kg, i.v.). Control WKY also received i.c.v. SSR240612. Raclopride alone was assessed in previous figures. Area under the curves (AUC) was measured for a period of 0-6 h (c, d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with aCSF values (*) or SSR240612 (†) is indicated by *, †*P*<0.05; **, ††*P*<0.01; ***, †††*P*<0.001.

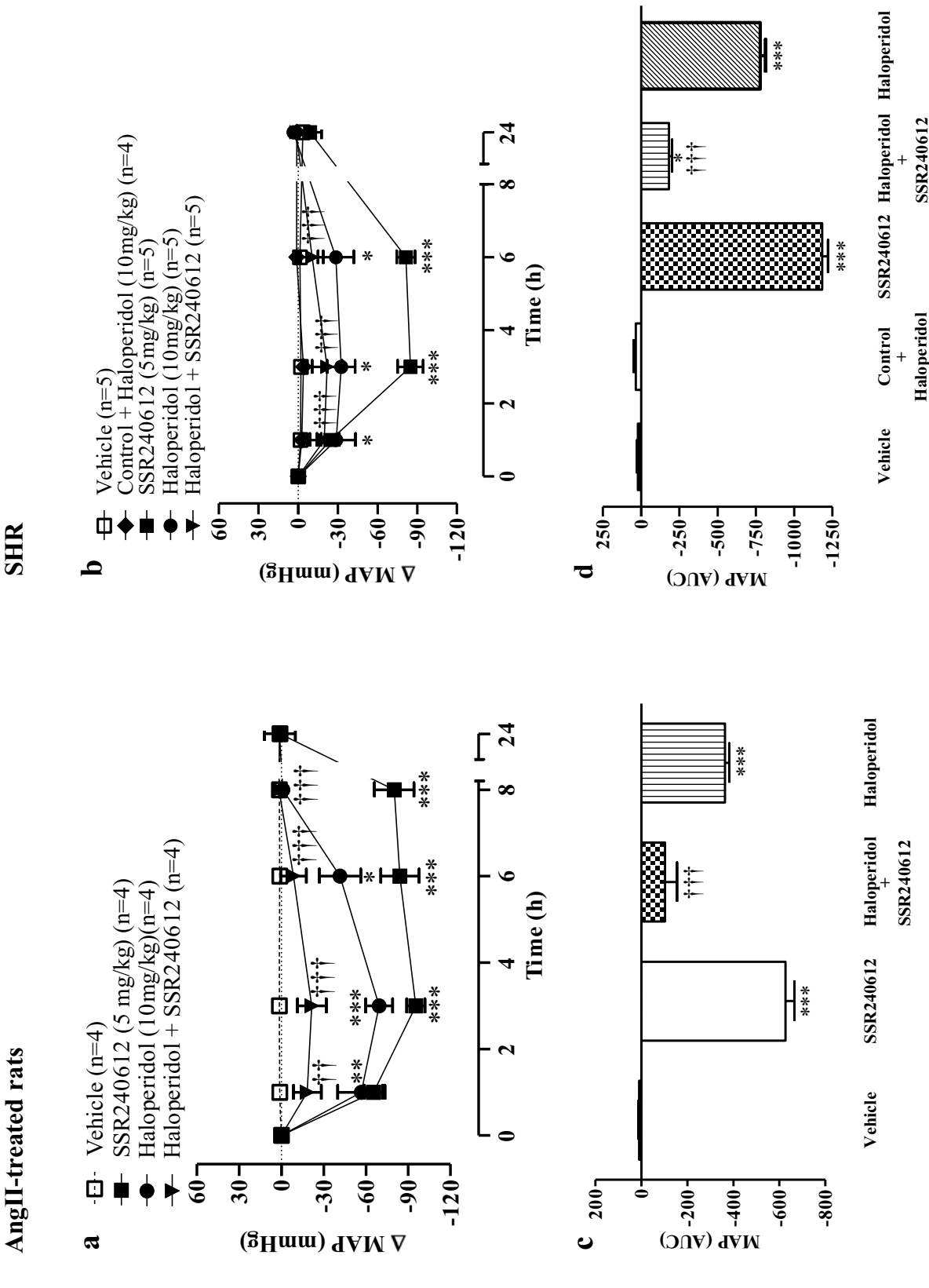
Fig.5: Time-course effects on change in mean arterial blood pressure (Δ MAP) induced by R-715 and R-954 (2mg/kg, s.c.) in AngII-treated rats (a) and SHR (b). Effects of both B1R antagonists were also tested in control WKY (a). Area under the curves (AUC) was measured for a period of 0-10 h (c) or 0-12 h (d). Data are means \pm s.e.m. of values obtained from (*n*) rats. Statistical comparison with vehicle values (*) is indicated by ***P*<0.01; ****P*<0.001.



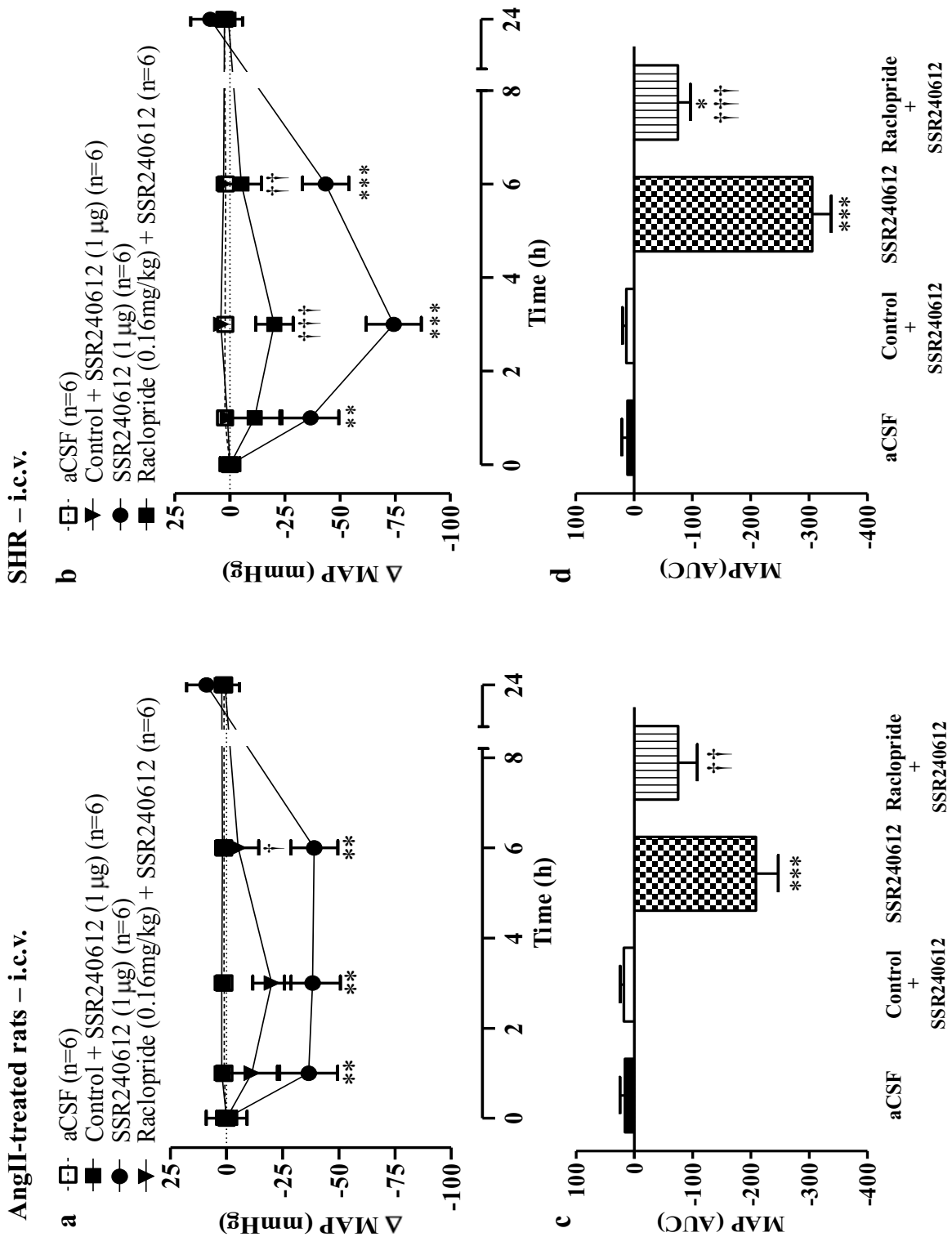
De Brito Gariepy
Fig.1



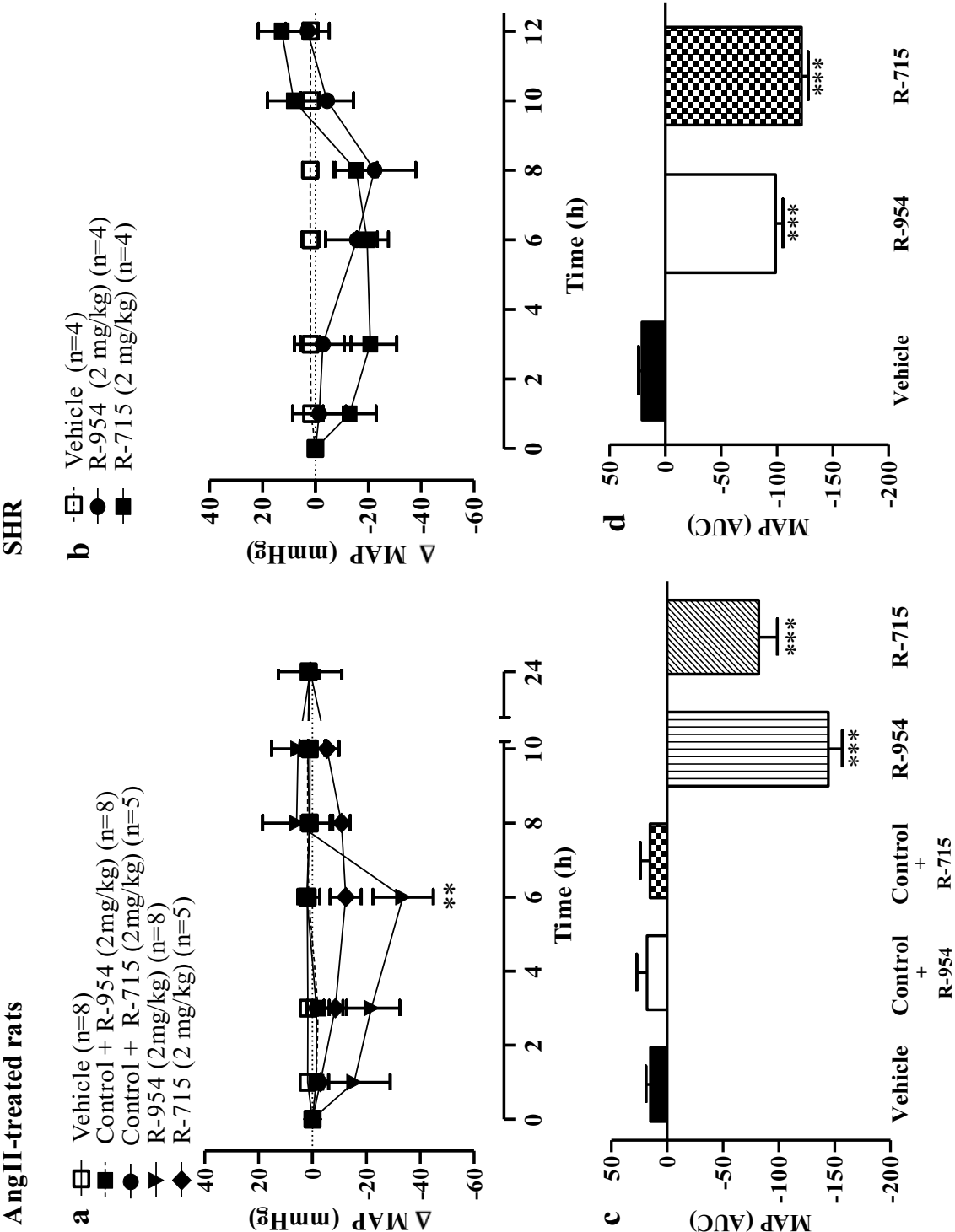
De Brito Gariepy
Fig.2



De Brito Gariepy
Fig.3



De Brito Gariepy
Fig.4



De Brito Garipey
Fig.5

12.3 **Article#3**

Contributions:

H. De Brito Garipey performed some of the experiments (Figures 2-4) and organized the results and wrote the manuscript. S. Talbot performed the mRNA experiments (Figure 1) and helped with redaction of the manuscript. R. Couture contributed in the redaction and revision of the manuscript.

**Mechanism underlying behavioural activity induced by activation of
brain kinin B1 receptor in hypertensive rats**

Helaine De Brito Gariépy, Sébastien Talbot and Réjean Couture*

Department of Physiology, Faculty of Medicine, Université de Montréal,
P.O. Box 6128, Succursale City Center, Montreal, QC, Canada - H3C 3J7

Running title: Kinin B1 receptor-induced stereotypic behaviour

Article in revision at British Journal Pharmacology, April 2010

*Author for correspondence:

Dr. Réjean Couture

Telephone: 1-514-343-7060

Fax: 1-514-343-2111

Summary

Background and purpose. Our laboratory recently reported a role for brain kinin B₁ receptor (B₁R) in the maintenance of hypertension in spontaneously hypertensive rat (SHR) and experimental hypertension induced by Angiotensin II (Ang II). This study aims at determining whether activation of B₁R can enhance behavioural activity in these two rat models of hypertension and at identifying the mechanism.

Experimental approach. SHR (16 weeks) and Ang II-hypertensive rats (200 ng·kg⁻¹ min⁻¹ × 2 weeks, s.c.) were i.c.v. injected with the selective B₁R agonist Sar[DPhe⁸][des-Arg⁹]BK (1 µg) before and after treatment with antagonists (10 µg i.c.v. or otherwise stated) for B₁ (SSR240612), tachykinin NK₁ (RP67580), glutamate NMDA (DL-AP5), dopamine D₁ (SCH23390, 0.2 mg·kg⁻¹ s.c.) and D₂ (Raclopride, 0.16 mg·kg⁻¹ s.c.) receptors or inhibitors (10 µg) of NOS (L-NNA) and iNOS (1400W). B₁R was also measured in selected brain areas by qRT-PCR. Wistar Kyoto rats (WKY) served as controls.

Key results. Whereas the B₁R agonist had no effect in WKY, it induced behavioural manifestations in both models of hypertension (face washing, sniffing, head scratching, rearing, teeth chattering, grooming, digging, licking, wet dog shake). These responses were prevented by all antagonists/inhibitors tested, while 1400W had a less inhibitory effect on most behaviors. Compared with controls, B₁R mRNA levels were markedly enhanced in hypothalamus, ventral tegmental area and nucleus accumbens of SHR and Ang II-treated rats.

Conclusion and implications: Activation of the upregulated cerebral kinin B₁R enhanced nocifensive behaviors in hypertensive rats through the release of several mediators, notably substance P, glutamate, dopamine and NO.

Keywords: Kinin B₁ receptor; tachykinin NK₁ receptor; behavior; dopamine; substance P; glutamate; nitric oxide

Abbreviations: Ang II, angiotensin II; aCSF, artificial cerebrospinal fluid; BK, bradykinin; DA, dopamine; DMSO, dimethylsulphoxide; NOS, nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; i.c.v., intracerebroventricular (ly); R, receptor; s.c., subcutaneous (ly); SP, substance P; SHR, spontaneously hypertensive rat; VTA, ventral tegmental area; WKY, Wistar Kyoto rat

Introduction

Kinins are vaso- and neuro- active regulatory peptides which exert central and peripheral effects through the activation of two G-protein-coupled receptors (R) denoted as B₁ and B₂ (Regoli *et al.*, 1998). Bradykinin (BK) and Lys-BK are the preferential agonists at the B₂R which is widely and constitutively expressed in various cell types. The C-terminal kininase I metabolites des-Arg⁹-BK and Lys-des-Arg⁹-BK are the active endogenous agonists at B₁R which is virtually absent or weakly expressed in healthy tissues. The B₁R is *de novo* synthesised under pathological situations (Calixto *et al.*, 2004; Rodi *et al.*, 2005). Several authors have reported that Angiotensin II (Ang II) enhances the expression of B₁R in cardiovascular tissues and in cultured vascular smooth muscle cells (Ceravolo *et al.*, 2007; Fernandes *et al.*, 2006; Kintsurashvili *et al.*, 2001). The induction of B₁R in vascular smooth muscle cells was ascribed to the activation of AT₁ receptor that enhances the production of reactive oxygen species and the subsequent activation of phosphatidylinositol-3 kinase and the transcriptional nuclear factor-kappa B (NF-κB) (Morand-Contant *et al.*, 2010). NF-κB is the transcription factor that allows the increased expression of B₁R (Leeb-Lundberg *et al.*, 2005). The induction of B₁R by Ang II is reminiscent of the increased B₁R mRNA levels observed in the heart and renal tissues of mice in renin/angiotensin-dependent renovascular hypertension (Duka *et al.*, 2001). Autoradiography studies have also documented elevated densities of B₁R binding sites in the spinal cord (Petcu *et al.*, 2005) and specific brain areas (substantia nigra, septum, posterodorsal tegmental nucleus and temporal cortex) (De Brito Pereira *et al.*, 2008) in the rat model of hypertension induced by a 2-week treatment with Ang II. Enhanced densities of B₁R binding sites were also reported by autoradiography in cortex, thalamus, amygdala, hippocampus and hypothalamus of 16-week old SHR (Cloutier *et al.*, 2004; de Wardener, 2001). This is consistent with the increased B₁R mRNA level in the hypothalamus of 12- to 13-week old SHR (Qadri *et al.*, 2002).

The present study was undertaken to determine whether the increased expression of B₁R binding sites are functional receptors in the brain of SHR and Ang II-hypertensive rats. This question was addressed by the measurement of changes of behavior induced by intracerebroventricular (i.c.v.) injection of Sar[DPhe⁸][des-Arg⁹]BK, a stable and selective B₁R agonist (Drapeau *et al.*, 1991). The putative contribution of DA and other central mediators (substance P (SP), glutamate, nitric oxide (NO)) in B₁R-induced behavioural activity was determined using a pharmacologic approach with selective receptor antagonists and enzymatic inhibitors. The increased expression of B₁R at mRNA level was also ascertained in selected brain regions related to behavior and the mesolimbic dopaminergic system.

Materials and Methods

Animal source and care

Male SHR (16 weeks) and age-matched Wistar Kyoto rats (WKY) were purchased one week prior to experiments from Charles River (St. Constant, Québec, Canada) and housed two per cage under pathogen-free conditions on a 12:12-h light-dark cycle in a room maintained at 22°C and 40% humidity. Food (Charles River Rodent) and tap water were accessible *ad libitum*. All research procedures and the care of the animals were in compliance with the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and were approved by the Animal Care Committee of our University.

Animal models and surgery

Under isoflurane anesthesia, WKY were implanted subcutaneously with a micro-osmotic pump (Alzet 2002, Alza Corporation, Palo Alto, CA, USA) to deliver Ang II (200 ng·kg⁻¹ min⁻¹ in saline) for 14 days as described previously (Petcu *et al.*, 2005). Control rats were sham-operated WKY. An i.c.v. guide cannula was implanted into the left lateral brain ventricle in isoflurane anesthetised WKY, SHR and 10-day Ang II-treated rats as described earlier (Cloutier *et al.*, 2004). During surgery, the animals received the antibiotics Trimethoprim and Sulphadiazine (Tribrissen 24%, 0.05 ml·kg⁻¹, s.c., Schering Canada Inc., Pointe Claire, Québec, Canada) and the analgesic Ketoprophen (Anafen, 10 mg·kg⁻¹, s.c., Merial Canada Inc., Baie d'Urfé, Québec, Canada). Thereafter, the rats were placed in custom-made plastic cages (L x W x H, 40 x 23 x 20 cm) and housed in the same controlled conditions. Experimental protocols were initiated 4-5 days after i.c.v. implantation in freely behaving rats. I.c.v. implantations were confirmed in all rats used in the study after *post-mortem* examination.

Measurement of behavior and i.c.v. injections

The behavioural activity was measured as previously reported in a quiet testing room where only the experimenter had access (Deschamps and

Couture, 2005; Picard *et al.*, 1994). Rats remained in their resident cage and the food and water were removed during the protocol. Briefly, during every consecutive period of 15 s, a score of 1 or 0 was given depending on whether the animal showed the specific type of behavior or not, whatever its frequency, intensity or duration during that period. Summation of scores for the first 30 min period following the i.c.v. injection provided the behavioural scores for face washing, sniffing, head scratching, digging, grooming, rearing, licking and teeth chattering. The maximal theoretical score was 120 (15 s intervals x 30 min). The wet dog shake was measured according to the number of episodes or frequency during the first 30 min period, whatever the intensity.

For i.c.v. injection, a 31GA stainless-steel injector was connected to the guide cannula. The other end was connected to a Hamilton microsyringe 5µl (Fisher Scientific Ltd, Montréal, Québec, Canada) with a PE-10 tubing. Injection was made i.c.v. in undisturbed freely behaving rats. The injector was removed from the guide cannula 1 min after injection to prevent any possible leakage of the injectate. All solutions were freshly prepared and injected in a volume of 1 µl over a period of 1 min.

Experimental protocols

In a first series of experiments, SHR, Ang II-treated rats and age-matched sham-operated rats received by i.c.v. a microinjection of artificial cerebrospinal fluid (aCSF) followed 60 min later by a single dose of Sar[DPhe⁸][des-Arg⁹]BK (1 µg or 10 µg). This protocol served at determining the behavioural effects of 1 and 10 µg of B₁R agonist in the two models of hypertension and their normotensive counterpart.

In a second series of experiments, SHR and Ang II-treated rats were separated in 8 groups of 6 rats treated as follows: Group 1, B₁R antagonist SSR240612 (10 µg i.c.v.); Group 2, SSR240612 (10 mg·kg⁻¹ per gavage); Group 3, tachykinin NK₁R antagonist RP67580 (10 µg i.c.v.); Group 4, glutamate NMDA receptor antagonist DL-AP5 (10 µg i.c.v.); Group 5, nitric

oxide synthase inhibitor L-NNA (10 µg i.c.v.); Group 6, inducible nitric oxide synthase inhibitor 1400W (10 µg i.c.v.); Group 7, dopamine D₁R antagonist SCH23390 (0.2 mg·kg⁻¹ s.c.); Group 8, dopamine D₂R antagonist Raclopride (0.16 mg·kg⁻¹ s.c.). These treatments were given 30 min prior to Sar[DPhe⁸][des-Arg⁹]BK (1 µg) or 3 h earlier (B₁R antagonist i.c.v. and gavage). The behavioural responses induced by Sar[DPhe⁸][des-Arg⁹]BK (1 µg) were compared in the absence (2h prior to treatments) and presence of antagonists or inhibitors.

Determination of brain B₁R mRNA levels by SYBR green-based quantitative RT-PCR

SHR, Ang II-treated and age-matched sham-operated rats not implanted with an i.c.v. cannula were decapitated after anesthesia under CO₂ inhalation. Brains were removed from the skull and rinsed in saline to remove any surface blood and placed in a glass plate. With the use of a surgical microscope, the hypothalamus, VTA and nucleus accumbens were micro-dissected as described by Palkovits *et al.*, (1998). About 10 mg of tissue were put in RNAlater stabilization reagent (QIAGEN, Valencia, CA, USA). RNA extraction and real-time PCR were performed as described elsewhere (Talbot *et al.*, 2009). For standardization and quantification, rat 18S was amplified simultaneously. The real-time PCR primer pairs used in this study were designed by Vector NTI software and presented in Table 1.

Drugs and solutions

The aCSF was purchased from Harvard Bioscience (Massachusetts, U.S.A.). Sar[DPhe⁸][des-Arg⁹]BK was synthesised at the Research Institute of Biotechnology, National Research Council of Canada and dissolved in aCSF. The B₁R antagonist SSR240612 [(2R)-2-(((3R)-3-(1,3-benzodioxol-5-yl)-3-[[[(6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl)amino]-3-(4-[[2R,6S)-2,6-dimethylpiperidiny]methyl]phenyl)-N-isopropyl-N-methylpropanamide hydrochloride] (Gougat *et al.*, 2004) was kindly provided by Dr. Pierre Carayon from Sanofi-Aventis R&D (Montpellier,

France). It was dissolved in dimethylsulphoxide (DMSO 0.5%), and then ethanol (5%) and Tween-80 (5%) were added in this sequence and completed with distilled water for gavage (10 mg·kg⁻¹ in a volume of 1 ml by 100 g of body weight) (Dias *et al.*, 2007). SSR240612 was dissolved in DMSO (0.5%) and ethanol (5%) in this sequence and completed with aCSF for i.c.v. administration. Tachykinin NK₁R antagonist, RP67580 [Imiro-1 (methoxy-2 phenyl)-2ethyl]-2 diphenyl-7.7 perhydroisoindolone-4-(3aR, 7aR) (Garret *et al.*, 1991) was purchased from Tocris Bioscience (Ellisville, MO, USA). Ang II, NOS inhibitor L-NNA (N^ω-Nitro-L-arginine) (Furfine *et al.*, 1993), iNOS inhibitor 1400W (Babu and Griffith, 1998), NMDA receptor antagonist DL-AP5 (DL-2-Amino-5-phosphonopentanoic acid) (Kent *et al.*, 1996), dopamine D₂R antagonist raclopride and dopamine D₁R antagonist SCH23390 (Millan *et al.*, 1998) were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada). Raclopride and SCH23390 were dissolved in DMSO and completed in saline (final solution contains 3% DMSO). All the other reagents were dissolved in aCSF. Drug and receptors nomenclature were cited conforms to BJP's Guide to Receptors and Channels (Alexander *et al.*, 2008).

Statistical analysis of data

Results are expressed as the means ± s.e. mean of values obtained from (n) rats. Statistical analysis of data was performed with Graph-Pad Prism software. B₁R mRNA levels were analyzed for statistical significance with Student's *t*-test for unpaired samples. Data of behaviors were analyzed with the non-parametric Kruskal-Wallis test (Figure 2) or the non-parametric Wilcoxon-Mann-Whitney test (Figures 3-4). Only probability values (P) less than 0.05 were considered to be statistically significant.

Results

Brain B₁R mRNA levels in SHR and Ang II-treated rats

Using quantitative real-time PCR, the overexpression of B₁R was confirmed in the brain of SHR and Ang II-treated rats (Fig. 1). B₁R mRNA levels were markedly increased in the hypothalamus, VTA and nucleus accumbens

isolated from both rat models of hypertension in comparison with age-matched WKY. The upregulation of B₁R in SHR was greater in intensity in the hypothalamus and VTA while in Ang II-treated rats the expression was greater in the VTA and nucleus accumbens.

Behavioural effects induced by kinin B₁R agonist in SHR and Ang II-treated rats

The first series of experiments showed that 1 and 10 µg Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR and Ang II-treated rats caused several behavioural manifestations while the same treatments in WKY had no significant effect when compared to aCSF (data not shown). In Fig. 2 the B₁R agonist have been injected into the i.c.v. in control WKY rats, which caused no behavioural activity when compared with B₁R injected into the i.c.v. in SHR. Doses of 1 µg and 10 µg Sar[DPhe⁸][des-Arg⁹]BK caused comparable effects and therefore the dose of 1 µg was selected for the remainder of the study. In SHR, 1 µg (i.c.v.) of the B₁R agonist caused significant increases of behavioural activity when compared to aCSF (face washing > sniffing = head scratching = rearing = teeth chattering >> grooming = digging > licking = wet dog shake) (Fig. 3a and c). In Ang II-treated rats, Sar[DPhe⁸][des-Arg⁹]BK (1 µg) injected i.c.v. also enhanced significantly behavioural activity but with a somewhat different pattern (sniffing ≥ teeth chattering > head scratching = grooming = rearing ≥ face washing > digging = licking = wet dog shake) (Fig. 3b and d).

Blockade of behavioural effects induced by kinin B₁R agonist in SHR

In SHR, the behavioural responses induced by 1 µg Sar[DPhe⁸][des-Arg⁹]BK were significantly blocked by the B₁R antagonist SSR240612 administered either i.c.v. (10 µg) or by gavage (10 mg·kg⁻¹) 3 h earlier (Fig. 3a and c). Teeth chattering and wet dog shake were reduced only when the B₁R antagonist was given i.c.v., suggesting that systemic SSR240612 reached less efficiently the brain area involved in these behaviors. Moreover, most stereotypic behaviors induced by 1 µg Sar[DPhe⁸][des-Arg⁹]BK in SHR were significantly blocked by the prior i.c.v. injection (10

µg, 30 min earlier) of the tachykinin NK₁R antagonist RP67580 (Fig. 4a), the glutamate NMDA receptor antagonist DL-AP5 (Fig. 4b), the NOS inhibitor L-NNA (Fig. 4c), the dopamine D₁R antagonist SCH23390 (Fig. 4e) and the dopamine D₂R antagonist Raclopride (Fig. 3f). Conversely, pre-treatment with the iNOS inhibitor 1400W (10 µg i.c.v. 30 min earlier) had a small inhibitory effect on most behaviors while digging and wet dog shake were not significantly affected (Fig. 3d). Whereas the level of inhibition achieved with 1400W was much less than that seen with the other treatments, face washing was almost abolished. When the B₁R agonist was re-injected alone 24h later, the behavioural manifestations were back to responses elicited in the absence of antagonists or inhibitors (data not shown). None of the antagonists or inhibitors caused behavioural changes per their own (data not shown).

Blockade of behavioural effects induced by kinin B₁R agonist in AngII-treated rats

In Ang II-treated rats, all behavioural manifestations evoked by 1 µg Sar[DPhe⁸][des-Arg⁹]BK were significantly blocked by SSR240612 administered either i.c.v. (Fig. 3b) or by gavage (Fig. 3d), 3 h earlier. Similarly to SHR, SSR240612 was more effective in inhibiting teeth chattering when injected i.c.v. As shown in Figure 5, behavioural activities elicited by Sar[DPhe⁸][des-Arg⁹]BK were significantly blocked by the tachykinin NK₁R antagonist RP67580, the glutamate NMDA receptor antagonist DL-AP5, the NOS inhibitor L-NNA, the dopamine D₁ and D₂ receptors antagonists SCH23390 and Raclopride. Similarly to SHR, behavioural responses to the B₁R agonist were less reduced by the iNOS inhibitor 1400W (Fig. 4d). Behavioural activities caused by Sar[DPhe⁸][des-Arg⁹]BK were completely recovered 24h later and all antagonists and inhibitors tested had no direct effect on behavior in Ang II-treated rats (data not shown).

Discussion

The present study provides the first pharmacological evidence that central kinin B₁R stimulation can enhance behavioural and locomotor activity through a mechanism involving SP, DA, glutamate and NO. This was shown in SHR and Ang II-treated rats, two rat models of hypertension that overexpressed B₁R in the hypothalamus, VTA and nucleus accumbens. In contrast, the B₁R is weakly expressed and physiologically inert in control WKY. These findings and particularly those obtained with SP and DA antagonists suggest that the mesolimbic dopaminergic system could be a strategic site for the behavioural manifestations induced by the B₁R agonist.

It is known that i.c.v. injection of SP increases locomotor activity, awareness, face washing, sniffing and grooming that are typical behaviors observed during the defence reaction (Tschöpe *et al.*, 1992; Unger *et al.*, 1988). They are also similar to behavioural arousal reaction induced by endogenous release of SP in the response to morphine withdrawal (Michaud and Couture, 2003) and physiological stress (Culman *et al.*, 1997). The centrally mediated behavioural effects of SP were blocked by the NK₁R antagonist RP67580 (Cellier *et al.*, 1999; Culman *et al.*, 1995; Picard *et al.*, 1994).

The implication of SP and NO in B₁R-induced behavioural manifestations is reminiscent of B₁R-induced thermal hyperalgesia which was associated with the release of sensory SP and the subsequent production of NO following NK₁R activation in the spinal dorsal horn of type 1 diabetic rats (Couture *et al.*, 2001). The activation of NK₁R in the rat spinal cord with endogenous and exogenous SP also evoked hyperalgesia in the tail-flick test through glutamate release and the subsequent activation of the NMDA receptor and NO production (Radhakrishnan *et al.*, 1995; Sun *et al.*, 2003; Yashpal *et al.*, 1991). Pharmacological blockade of NOS prevented the responses to NMDA, SP and noxious cutaneous stimuli in cat dorsal horn (Radhakrishnan *et al.*, 1993).

Hence, one can suggest that a similar event occurs in the brain of SHR and Ang II-treated rats where the release of SP following B₁R activation triggers the release of glutamate and the activation of NMDA receptor. In this scenario, NO would be located downstream to mediate the final effect of the B₁R agonist on behavior. Data suggest that iNOS is not the primary source of NO for most behaviours elicited by the B₁R agonist.

In vitro studies have raised the possibility that cerebrovascular NO systems were altered in hypertensive rats (Miyata *et al.*, 1990; Malinski *et al.*, 1993). Moreover, perturbations in endothelium-dependent relaxation have been identified in pial arteries, which were examined *in situ* studies (Yang *et al.*, 1991; Mayhan, 1992). In contrast, *in vivo* studies showed that the cerebrovascular sensitivity to NO inhibition was conserved (Izuta *et al.*, 1995). Furthermore, basal local cerebral blood flow appears to be unaffected by hypertension (Wei *et al.*, 1992). Moreover, i.c.v. injections of high doses of NG-monomethyl-L-arginine (L-NMMA), a non selective NOS inhibitor, which can block eNOS, caused marked insulin resistance, hyperglycemia, defective insulin secretion, and hypertension (Skankar *et al.*, 1998). However, acute systemic induction of hypertension with L-NMMA may cause increased insulin resistance (Baron *et al.*, 1995). NO derived from eNOS located in endothelial cells of the brain may diffuse to nearby neurons and influence their activity. This notion is supported by data demonstrating the importance of NO derived from eNOS in the control of inhibitory neurotransmitter release in mouse cerebral cortex (Kano *et al.*, 1998).

Studies in aggressive behavior were analyzed using knockout mice, which are extremely aggressive, because of the disruption of the gene of nNOS. The results showed that nNOS is a major mediator of the aggressive behavior. The administration of nNOS inhibitor in wild-type mice increased the aggression behavior, suggesting that nNOS activators may have therapeutic roles in inflammatory, cardiovascular, and neurologic diseases (Demas *et al.*, 1997). However, the mechanism for the regulation of behavior by NOS in the brain is still unknown.

Behavioural activity induced by SP was also related to midbrain DA release (Stoessl *et al.*, 1991) which may suggest that the release of SP following B₁R activation can affect DA neurotransmission and account for the inhibitory effect of D₁R and D₂R antagonists on centrally mediated behavioural effects of B₁R agonist. This is keeping with the involvement of DA in the anti-hypertensive effect of B₁R antagonist in SHR and Ang II-treated rats (De Brito Gariépy *et al.*, 2010) and with the increased central dopaminergic activity reported in SHR (van den Buuse, 1991; Amenta *et al.*, 2001). Thus the activation of DA neurons through B₁R can be direct or indirect through the release of other central mediators such as SP. This is feasible because SP immunoreactive axon terminals make direct synaptic contact with DA neurons in the VTA (Tamiya *et al.*, 1990) on which NK₁R was localized by electron microscopy (Lessard *et al.*, 2009). Furthermore, the application of SP and NK₁R agonists into the VTA increased firing rate of A10 dopamine cells, the levels of DA and its metabolite dihydroxyphenylacetic acid (DOPAC) and DA turnover in the prefrontal cortex and nucleus accumbens (Cador *et al.*, 1989; Elliott *et al.*, 1986; Overton *et al.*, 1992). This was accompanied by increased behavioural activity consistent with mesolimbic DA activation (Deschamps and Couture, 2005; Eison *et al.*, 1982; Elliott *et al.*, 1986).

Since the mesolimbic dopaminergic pathway and SP are both involved in the defence reaction to physiological stress and peripheral noxious stimulation (Culman *et al.*, 2010; Culman *et al.*, 1997; Le Moal and Simon, 1991; van den Buuse, 1998), it is tempting to suggest that kinin B₁R may exert a central regulatory function in the coordination of nocifensive behavior and locomotor activity in response to stress and noxious stimulation. Although this hypothesis remains to be proven, it would be in line with the pro-nociceptive effect of B₁R in the periphery (Calixto *et al.*, 2004; Couture *et al.*, 2001).

Conclusion

The cerebral injection of a kinin B₁R agonist induces stereotyped behaviors and locomotor activity in SHR and Ang II-treated rats, two models of hypertension associated with a marked induction of B₁R in the hypothalamus, VTA and nucleus accumbens. The B₁R-induced behavioural responses are mediated by several central mediators, notably SP (NK₁ receptor), dopamine (D₁ and D₂ receptors), glutamate (NMDA receptor) and nitric oxide. Hence, cerebral activation of B₁R reproduced the nocifensive defence reaction induced by these mediators in the mesolimbic system.

Conflict of interested

The authors have declared that no competing interests exist.

Acknowledgements

This work was supported by a Grant-in-aid from the Canadian Institutes of Health Research (CIHR) (MOP-79471). H. De Brito Gariépy held a Studentship from the National Council of Technological and Scientific Development (CNPq) (proc. 201428/2003-2) Brazil. S. Talbot holds a Frederick Banting and Charles Best Canada Graduate Scholarships-Doctoral Award from CIHR. Authors are thankful to Mr Julien Saint-Denis for his technical assistance in qRT-PCR.

Table 1: PCR primer pairs used in this study

	Sequences			Position			Gen Bank
18S forward	5'	TCA ACT TTC GAT GGT AGT CGC CGT	3'	363	-	386	X01117
18S reverse	5'	TCC TTG GAT GTG GTA GCC GTT TCT	3'	470	-	447	
B ₁ receptor forward	5'	GCA GCG CTT AAC CAT AGC GGA AAT	3'	367	-	391	NM_030851
B ₁ receptor reverse	5'	CCA GTT GAA ACG GTT CCC GAT GTT	3'	478	-	454	

References

- Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edition. *Br J Pharmacol* **153 Suppl 2**: S1-209.
- Amenta F, Ricci A, Rossodivita I, Avola R, Tayebati SK (2001). The dopaminergic system in hypertension. *Clin Exp Hypertens* **23**(1-2): 15-24.
- Babu BR, Griffith OW (1998). Design of isoform-selective inhibitors of nitric oxide synthase. *Curr Opin Chem Biol* **2**(4): 491-500.
- Baron AD, Zhu J, Marshall S, Irsula O, Brechtel G, Keech C (1995). Insulin resistance following acute induction of hypertension with the nitric oxide synthesis inhibitor L-NMMA in rats. *Am J Physiol* **269**:E709-E715.
- Cador M, Rivet JM, Kelley AE, Le Moal M, Stinus L (1989). Substance P, neurotensin and enkephalin injections into the ventral tegmental area: comparative study on dopamine turnover in several forebrain structures. *Brain Res* **486**(2): 357-363.
- Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM (2004). Kinin B1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes. *Br J Pharmacol* **143**(7): 803-818.
- Cellier E, Barbot L, Iyengar S, Couture R (1999). Characterization of central and peripheral effects of septide with the use of five tachykinin NK1 receptor antagonists in the rat. *Br J Pharmacol* **127**(3): 717-728.
- Ceravolo GS, Fernandes L, Munhoz CD, Fernandes DC, Tostes RC, Laurindo FR, *et al.* (2007). Angiotensin II chronic infusion induces B1 receptor expression in aorta of rats. *Hypertension* **50**(4): 756-761.

Cloutier F, Ongali B, Campos MM, Thibault G, Neugebauer W, Couture R (2004). Correlation between brain bradykinin receptor binding sites and cardiovascular function in young and adult spontaneously hypertensive rats. *Br J Pharmacol* **142**(2): 285-296.

Couture R, Harrisson M, Vianna RM, Cloutier F (2001). Kinin receptors in pain and inflammation. *Eur J Pharmacol* **429**(1-3): 161-176.

Culman J, Das G, Ohlendorf C, Haass M, Maser-Gluth C, Zuhayra M, Zhao Y, Itoi K (2010). Blockade of tachykinin NK(1)/NK(2) receptors in the brain attenuates the activation of CRH-neurons in the hypothalamic paraventricular nucleus and the sympathoadrenal and pituitary-adrenal responses to formalin induced pain in the rat. *J Neuroendocrinol*. 2010 [Epub ahead of print]

Culman J, Klee S, Ohlendorf C, Unger T (1997). Effect of tachykinin receptor inhibition in the brain on cardiovascular and behavioural responses to stress. *J Pharmacol Exp Ther* **280**(1): 238-246.

Culman J, Wiegand B, Spitznagel H, Klee S, Unger T (1995). Effects of the tachykinin NK1 receptor antagonist, RP 67580, on central cardiovascular and behavioural effects of substance P, neurokinin A and neurokinin B. *Br J Pharmacol* **114**(6): 1310-1316.

De Brito Pereira H, Talbot S, Ongali B, Couture R (2008). Role of kinin B1 receptor in the central autonomic control of blood pressure and body temperature in two rat models of hypertension. In: 39th Annual Meeting of the Society for Neuroscience, November 12-15, Washington, DC, USA (P83.18).

De Brito Gariepy H, Carayon P, Ferrari B, Couture R (2010). Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin

B1 receptor antagonists in two rat models of hypertension. *Neuropeptides* **44**(2):191-8.

Demas GE, Eliasson MJ, Dawson TN, Dawson TM, Kriegsfeld LJ, Nelson RJ, Snyder SH (1997). Inhibition of neuronal nitric oxide synthase increases aggressive behavior in mice. *Mol Med* **3**(9): 610-616.

de Wardener HE (2001). The hypothalamus and hypertension. *Physiol Rev* **81**(4): 1599-1658.

Deschamps K, Couture R (2005). The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br J Pharmacol* **145**(6): 712-727.

Dias JP, Ismael MA, Pilon M, de Champlain J, Ferrari B, Carayon P, *et al.* (2007). The kinin B1 receptor antagonist SSR240612 reverses tactile and cold allodynia in an experimental rat model of insulin resistance. *Br J Pharmacol* **152**(2): 280-287.

Drapeau G, deBlois D, Marceau F (1991). Hypotensive effects of Lys-des-Arg9-bradykinin and metabolically protected agonists of B1 receptors for kinins. *J Pharmacol Exp Ther* **259**(3): 997-1003.

Duka I, Kintsurashvili E, Gavras I, Johns C, Bresnahan M, Gavras H (2001). Vasoactive potential of the b(1) bradykinin receptor in normotension and hypertension. *Circ Res* **88**(3): 275-281.

Eison AS, Eison MS, Iversen SD (1982). The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area or substantia nigra of rat brain. *Brain Res* **238**(1): 137-152.

Elliott PJ, Alpert JE, Bannon MJ, Iversen SD (1986). Selective activation of mesolimbic and mesocortical dopamine metabolism in rat brain by

infusion of a stable substance P analogue into the ventral tegmental area. *Brain Res* **363**(1): 145-147.

Fernandes L, Ceravolo GS, Fortes ZB, Tostes R, Santos RA, Santos JA, *et al.* (2006). Modulation of kinin B1 receptor expression by endogenous angiotensin II in hypertensive rats. *Regul Pept* **136**(1-3): 92-97.

Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel JF, Blanchard JC, *et al.* (1991). Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci U S A* **88**(22): 10208-10212.

Gougat J, Ferrari B, Sarrao L, Planchenault C, Poncelet M, Maruani J, *et al.* (2004). SSR240612 [(2R)-2-[[[(3R)-3-(1,3-benzodioxol-5-yl)-3-[[[(6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl]amino]-3-(4-[[[2R,6S)-2,6-dimethylpiperidinyl]methyl]phenyl]-N-isopropyl-N-methylpropanamide hydrochloride], a new nonpeptide antagonist of the bradykinin B1 receptor: biochemical and pharmacological characterization. *J Pharmacol Exp Ther* **309**(2): 661-669.

Izuta M, Clavier N, Kirsch JR, Traystman RJ (1995). Cerebral blood flow during inhibition of brain nitric oxide synthase activity in normal, hypertensive, and stroke-prone rats. *Stroke* **26**:1079 ± 1085.

Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA, Lo EH (1998). Effects of nitric oxide synthase gene knockout on neurotransmitter release in vivo. *Neuroscience* **86**:695– 699.

Kent S, Kernahan SD, Levine S (1996). Effects of excitatory amino acids on the hypothalamic-pituitary-adrenal axis of the neonatal rat. *Brain Res Dev Brain Res* **94**(1): 1-13.

Kintsurashvili E, Duka I, Gavras I, Johns C, Farmakiotis D, Gavras H (2001). Effects of ANG II on bradykinin receptor gene expression in

cardiomyocytes and vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* **281**(4): H1778-1783.

Le Moal M, Simon H (1991). Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol Rev* **71**(1): 155-234.

Leeb-Lundberg LM, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL (2005). International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev* **57**(1): 27-77.

Lessard A, Savard M, Gobeil F, Jr., Pierce JP, Pickel VM (2009). The neurokinin-3 (NK3) and the neurokinin-1 (NK1) receptors are differentially targeted to mesocortical and mesolimbic projection neurons and to neuronal nuclei in the rat ventral tegmental area. *Synapse* **63**(6): 484-501.

Malinski T, Kapturczak M, Dayharsh J, Borh D (1993). Nitric oxide synthase activity in genetic hypertension. *Biochem Biophys Res Commun* **194**:654 ± 658.

Mayhan WG (1992). Role of prostaglandin H₂-thromboxane A₂ in responses of cerebral arterioles during chronic hypertension. *Am J Physiol* **262**:H539 ±H543.

Michaud N, Couture R (2003). Cardiovascular and behavioural effects induced by naloxone-precipitated morphine withdrawal in rat: characterization with tachykinin antagonists. *Neuropeptides* **37**(6): 345-354.

Millan MJ, Newman-Tancredi A, Brocco M, Gobert A, Lejeune F, Audinot V, *et al.* (1998). S 18126 ([2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl methyl]indan-2-yl]), a potent, selective and competitive antagonist at dopamine D₄ receptors: an in vitro and in vivo comparison with L 745,870

(3-(4-[4-chlorophenyl]piperazin-1-yl)methyl-1H-pyrrolo[2, 3b]pyridine) and raclopride. *J Pharmacol Exp Ther* **287**(1): 167-186.

Miyata N, Tsuchida K, Tanaka M, Otomo S (1990). Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in carotid arteries from stroke-prone spontaneously hypertensive rats. *J Pharm Pharmacol* **42**: 763 ± 766.

Morand-Contant M, Anand-Srivastava, MB, Couture R (2010). Kinin B₁ receptor upregulation by angiotensin II and endothelin-1 in rat vascular smooth muscle cells: Receptors and mechanisms. *Am J Physiol*. (In revision).

Overton P, Elliott PJ, Hagan RM, Clark D (1992). Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. *Eur J Pharmacol* **213**(1): 165-166.

Palkovits M, Young WS, 3rd, Kovacs K, Toth Z, Makara GB (1998). Alterations in corticotropin-releasing hormone gene expression of central amygdaloid neurons following long-term paraventricular lesions and adrenalectomy. *Neuroscience* **85**(1): 135-147.

Petcu M, Ongali B, El Midaoui A, de Champlain J, Couture R (2005). Effects of alpha-lipoic acid on kinin B₁ and B₂ receptor binding sites in the spinal cord of chronically angiotensin-treated rats. *Peptides* **26**(8): 1331-1338.

Picard P, Regoli D, Couture R (1994). Cardiovascular and behavioural effects of centrally administered tachykinins in the rat: characterization of receptors with selective antagonists. *Br J Pharmacol* **112**(1): 240-249.

Qadri F, Hauser W, Jöhren O, Dominiak P (2002). Kinin B₁ and B₂ receptor mRNA expression in the hypothalamus of spontaneously hypertensive rats. *Can J Physiol Pharmacol* **80**(4): 258-263.

Radhakrishnan V, Henry JL (1993). L-NAME blocks responses to NMDA, substance P and noxious cutaneous stimuli in cat dorsal horn. *Neuroreport* **4**(3): 323-326.

Radhakrishnan V, Yashpal K, Hui-Chan CW, Henry JL (1995). Implication of a nitric oxide synthase mechanism in the action of substance P: L-NAME blocks thermal hyperalgesia induced by endogenous and exogenous substance P in the rat. *Eur J Neurosci* **7**(9): 1920-1925.

Regoli D, Nsa Allogho S, Rizzi A, Gobeil FJ (1998). Bradykinin receptors and their antagonists. *Eur J Pharmacol* **348**(1): 1-10.

Rodi D, Couture R, Ongali B, Simonato M (2005). Targeting kinin receptors for the treatment of neurological diseases. *Curr Pharm Des* **11**(10): 1313-1326.

Shankar R, Zhu JS, Ladd B, Henry D, Shen HG, Baron AD (1998). Central Nervous System Nitric Oxide Synthase Activity Regulates Insulin Secretion and Insulin Action. *J Clin Invest* **102**(7): 1403-1412.

Stoessl AJ, Szczutkowski E, Glenn B, Watson I (1991). Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. *Brain Res* **565**(2): 254-262.

Sun XC, Li WB, Li SQ, Li QJ, Chen XL, Ai J (2003). [Intrathecal injection of Sar9, Met(O²)11-substance P, neurokinin-1 receptor agonist, increases nitric oxide synthase expression and nitric oxide production in the rat spinal cord]. *Sheng Li Xue Bao* **55**(6): 677-683.

Talbot S, Theberge-Turmel P, Liazoghli D, Senecal J, Gaudreau P, Couture R (2009). Cellular localization of kinin B1 receptor in the spinal cord of

streptozotocin-diabetic rats with a fluorescent [Nalpha-Bodipy]-des-Arg9-bradykinin. *J Neuroinflammation* **6**: 11.

Tamiya R, Hanada M, Kawai Y, Inagaki S, Takagi H (1990). Substance P afferents have synaptic contacts with dopaminergic neurons in the ventral tegmental area of the rat. *Neurosci Lett* **110**(1-2): 11-15.

Tschope C, Picard P, Culman J, Prat A, Itoi K, Regoli D, *et al.* (1992). Use of selective antagonists to dissociate the central cardiovascular and behavioural effects of tachykinins on NK1 and NK2 receptors in the rat. *Br J Pharmacol* **107**(3): 750-755.

Unger T, Carolus S, Demmert G, Ganten D, Lang RE, Maser-Gluth C, *et al.* (1988). Substance P induces a cardiovascular defense reaction in the rat: pharmacological characterization. *Circ Res* **63**(4): 812-820. van den Buuse M, Linthorst AC, Versteeg DH, de Jong W (1991). Role of brain dopamine systems in the development of hypertension in the spontaneously hypertensive rat. *Clin Exp Hypertens* **13**(5):653-9.

van den Buuse M (1998). Role of the mesolimbic dopamine system in cardiovascular homeostasis. Stimulation of the ventral tegmental area modulates the effect of vasopressin on blood pressure in conscious rats. *Clin Exp Pharmacol Physiol* **25**(9): 661-668.

Wei L, Lin SZ, Tajima A, Nakata H, Acuff V, Patlak C, Pattigrew K, Fenstermacher J (1992). Cerebral glucose utilization and blood flow in adult spontaneously hypertensive rats. *Hypertension* **20**:501 ± 510.

Yashpal K, Radhakrishnan V, Henry JL (1991). NMDA receptor antagonist blocks the facilitation of the tail flick reflex in the rat induced by intrathecal administration of substance P and by noxious cutaneous stimulation. *Neurosci Lett* **128**(2): 269-272.

Yang ST, Mayhan WG, Faraci FM, Heistad DD (1991). Endothelium-dependent responses of cerebral blood vessels during chronic hypertension. *Hypertension* **17**: 612 ± 618.

Figure legends

Figure 1. B₁R gene expression in hypothalamus, ventral tegmental area (VTA) and nucleus accumbens of SHR and Ang II-treated rats. B₁R mRNA levels were measured relative to 18S level. Data represent the mean ± s.e.mean of values obtained in groups of 4 rats. Statistical comparison to control WKY is indicated by * P < 0.05; ** P < 0.01 or ***P < 0.01.

Figure 2. Behavioural responses to 1 µg Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR and WKY. Each bar represents the mean ± s.e.mean of values obtained in 4 rats per group. Comparison to WKY is indicated by ***P < 0.001.

Figure 3. Inhibition of the behavioural responses to 1 µg Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR (a, c) and Ang II-treated rats (b, d) before and 3h after treatment with the kinin B₁R antagonist SSR240612 administered either i.c.v. (10 µg) or by gavage (10 mg·kg⁻¹). Each bar represents the mean ± s.e.mean of values obtained in 4 groups of 6 rats. Comparison to aCSF (*) and to the B₁R agonist (†) is indicated by *† P < 0.05; **†† P < 0.01; ***P < 0.001.

Figure 4. Inhibition of the behavioural responses to 1 µg Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR before and 30 min after treatment with the tachykinin NK₁R antagonist RP 67580 (a), the NMDA receptor antagonist DL-AP5 (b), the NOS inhibitor L-NNA (c), the iNOS inhibitor 1400W (d), the dopamine D₁R antagonist SCH23390 (e), the dopamine D₂R antagonist Raclopride (f). Each bar represents the mean ± s.e.mean of values obtained

in 6 groups of 6 rats. Comparison to the B₁R agonist before treatment is indicated by ***P** < 0.05, ****P** < 0.01.

Figure 5. Inhibition of the behavioural responses to 1 µg Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in Ang II-treated rats before and 30 min after treatment with the tachykinin NK₁R antagonist RP 67580 (a), the NMDA receptor antagonist DL-AP5 (b), the NOS inhibitor L-NNA (c), the iNOS inhibitor 1400W (d), the dopamine D₁R antagonist SCH23390 (e), the dopamine D₂R antagonist Raclopride (f). Each bar represents the mean ± s.e.mean of values obtained in 6 groups of 6 rats. Comparison to the B₁R agonist before treatment is indicated by ***P** < 0.05, ****P** < 0.01.

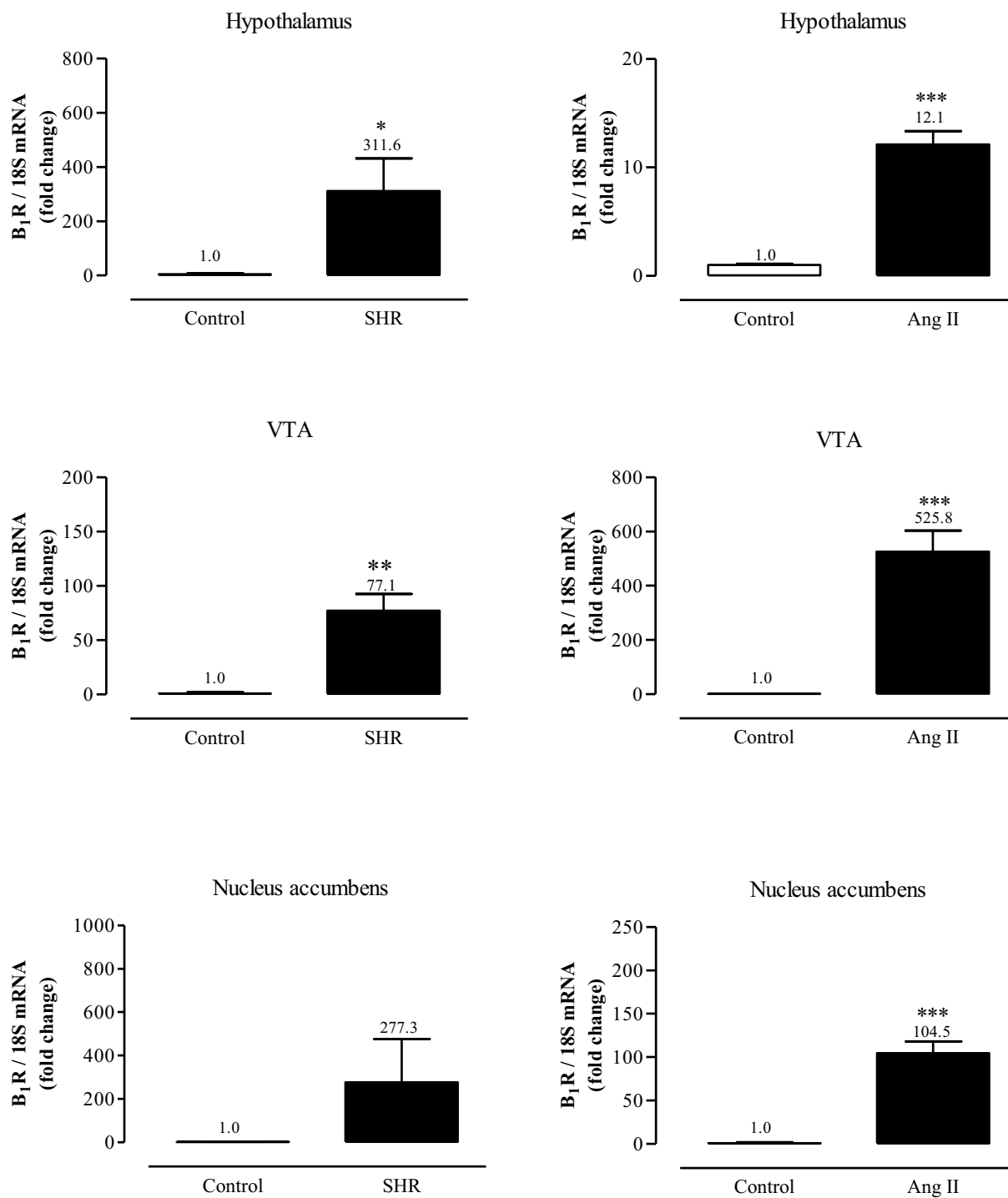


Figure 1.
De Brito Gariepy et al.

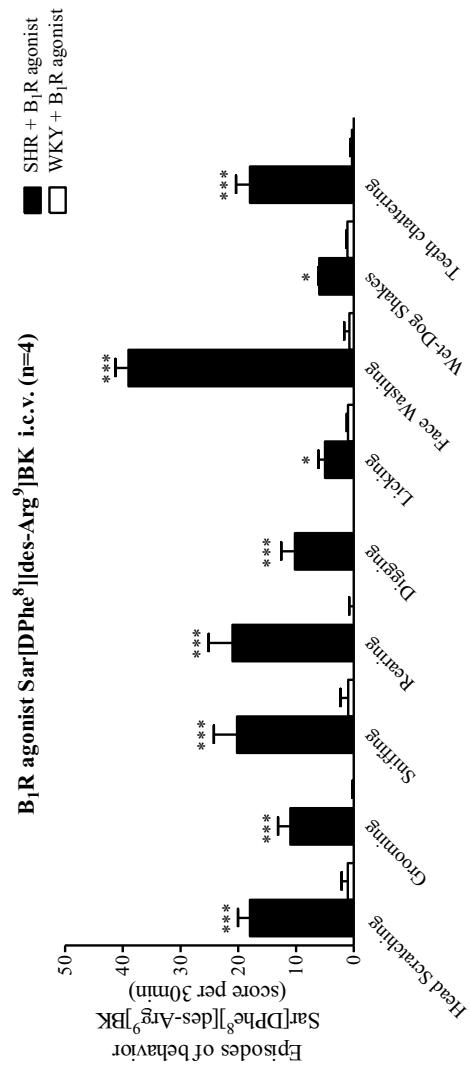


Figure 2
De Brito Gariepy et al.

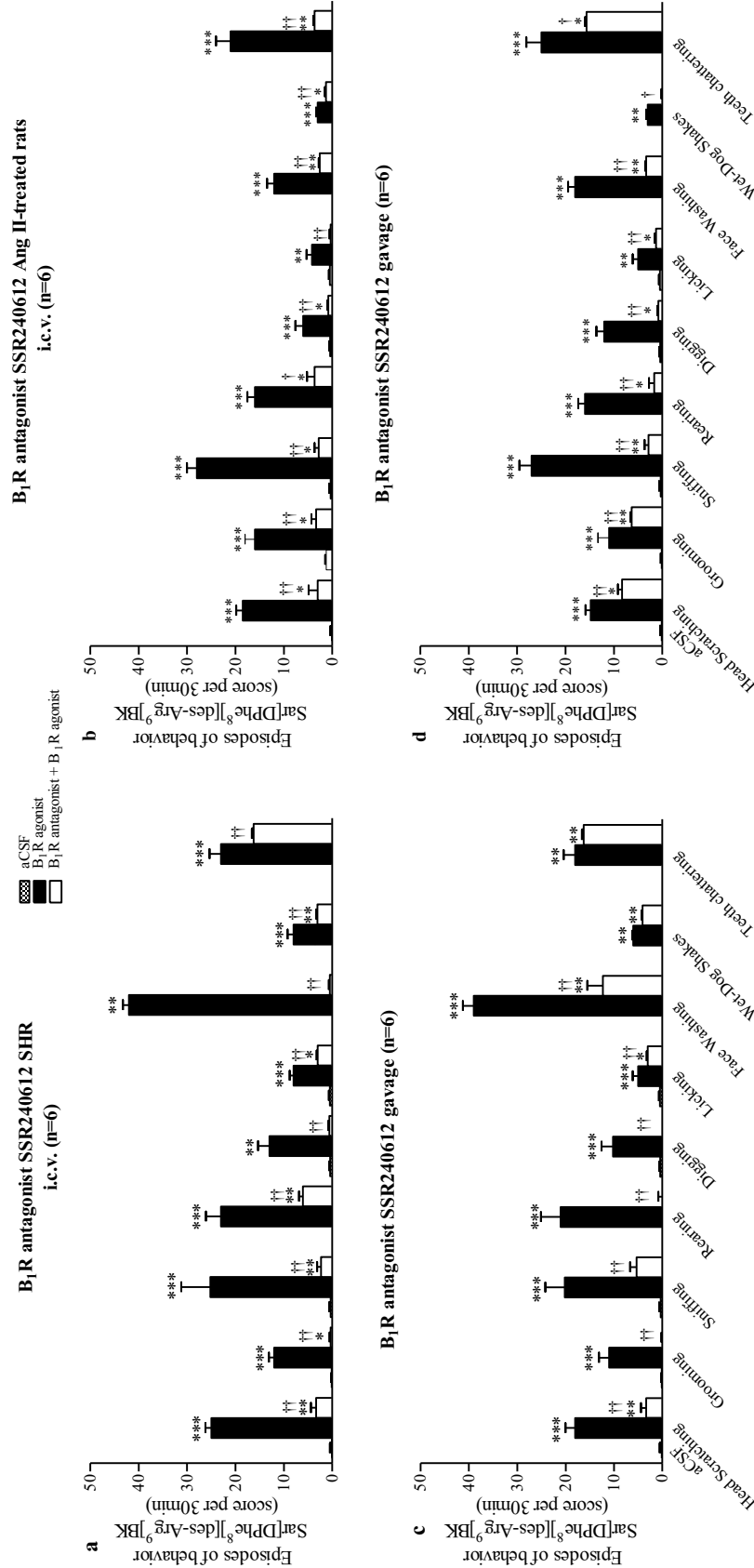


Figure 3
De Brito Garipey et al.

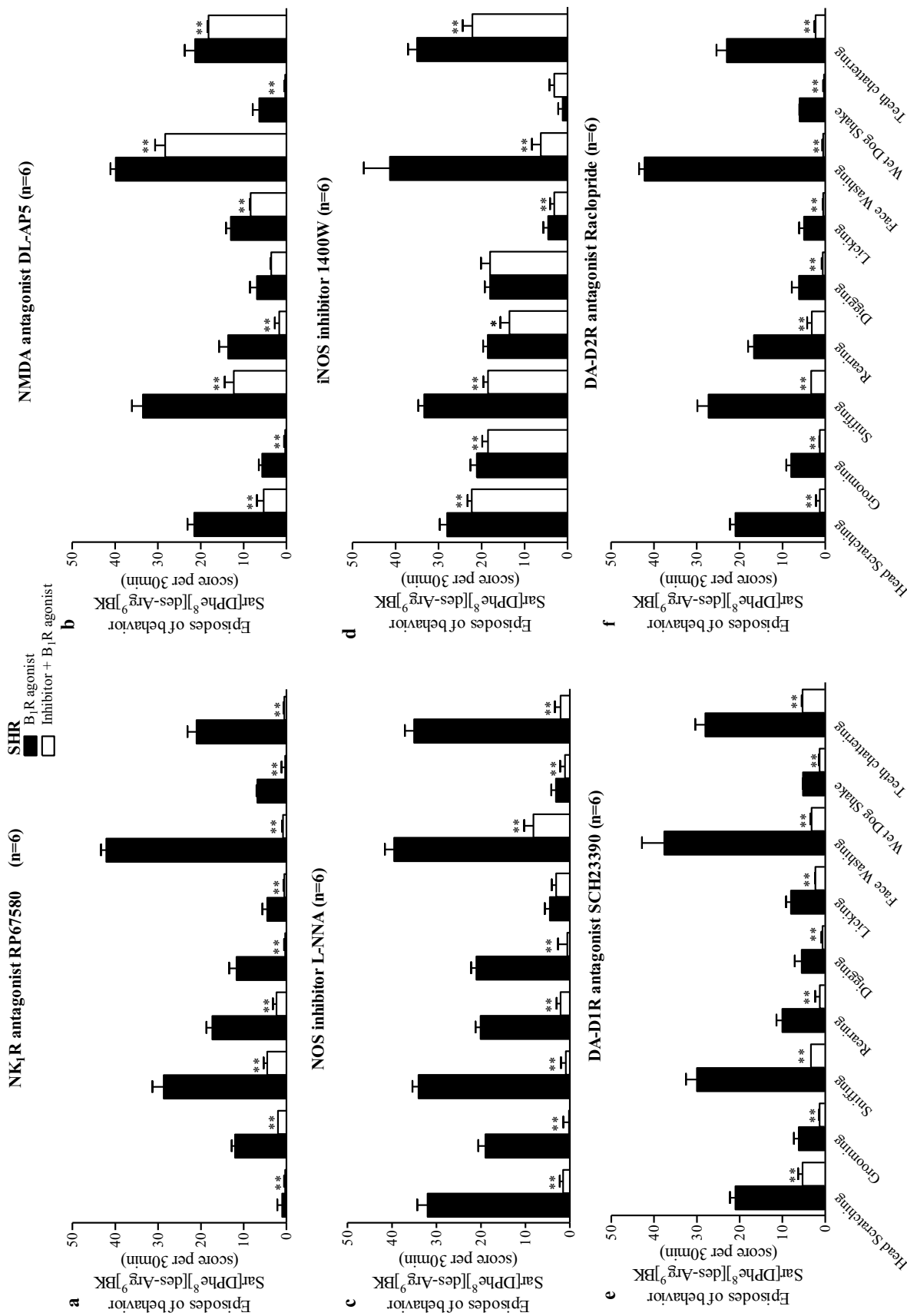


Figure 4
De Brito Garipey et al.

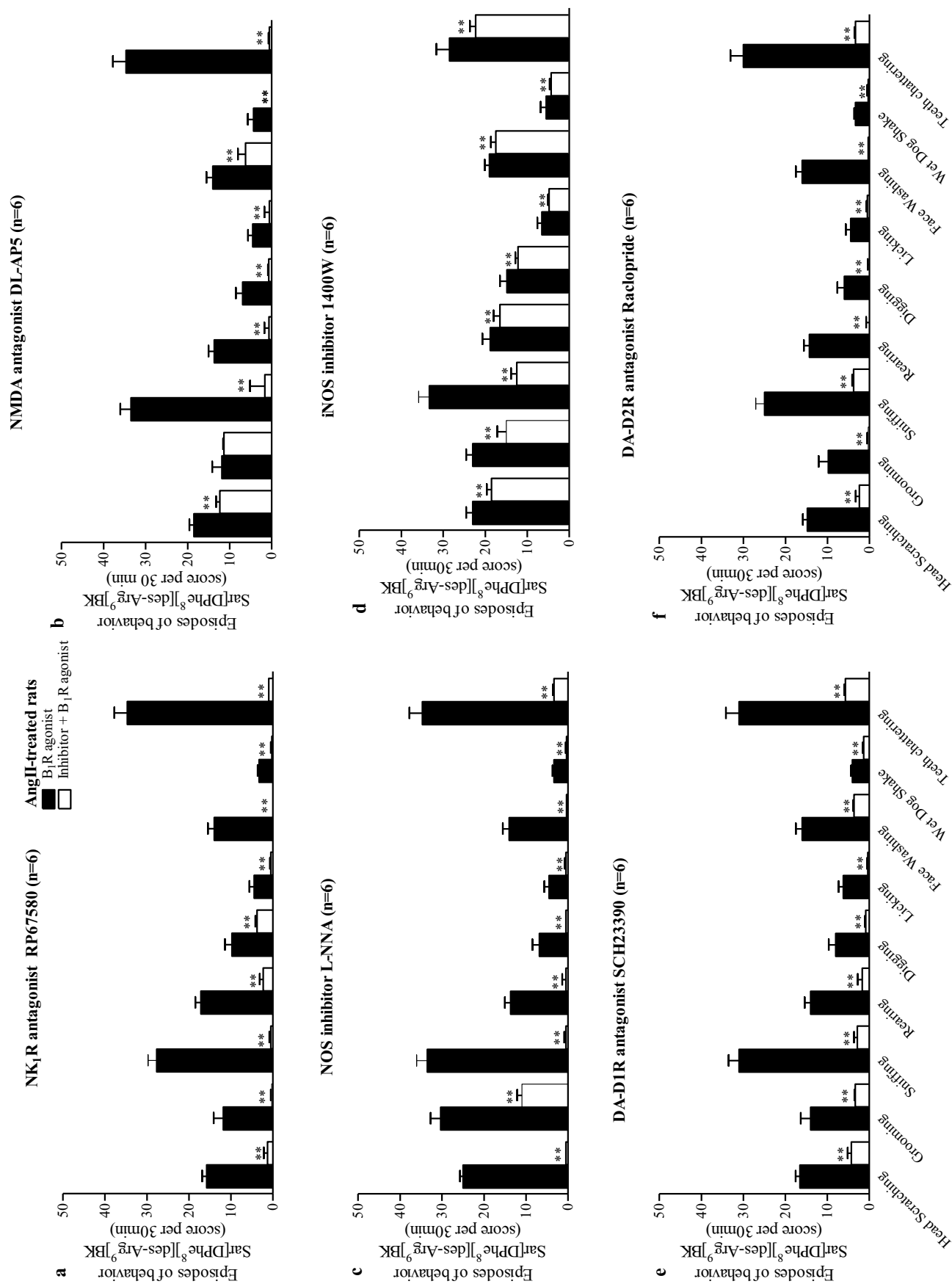


Figure 5
De Brito Garipey et al.

Chapter IV

General Discussion and General Conclusion

13. General Discussion

13.1 Central tachykinin NK-3R in hypertension

The first study described in the Chapter I was a continuation of a previous study from our laboratory showing that tachykinin NK-3R antagonists exert dose-dependent and reversible anti-hypertensive effects when injected i.c.v. in SHR (Lessard *et al.*, 2004). The objective of this thesis was to further define the site of action and the mechanism responsible for this central anti-hypertensive effect in SHR and to determine whether the blockade of kinin B1R can reproduce this effect based on the finding that this receptor is induced and overexpressed in the CNS of hypertensive rats. The dopaminergic pathway in the VTA was studied as a putative target for tachykinin NK-3R and kinin B1R antagonists based on the previous review of the literature.

The thesis shows firstly the effect of the tachykinin NK-3R agonist senktide when injected either i.c.v. or directly into the VTA in SHR (Figure 12). Previous studies have documented the effects of i.c.v. and VTA injected senktide in normotensive control rats (Cellier *et al.*, 1997; Deschamps and Couture, 2005). In SHR, senktide caused maximal increases of BP and HR at the dose of 25 pmol when injected into the VTA as reported previously in Wistar rats (Deschamps and Couture, 2005), while the maximal effect of senktide injected i.c.v. was achieved at the dose of 65 pmol in SHR and at 1 nmol in Wistar rats (Cellier *et al.*, 1997). Senktide-induced increases of BP and HR were also observed in guinea-pig (Roccon *et al.*, 1996).

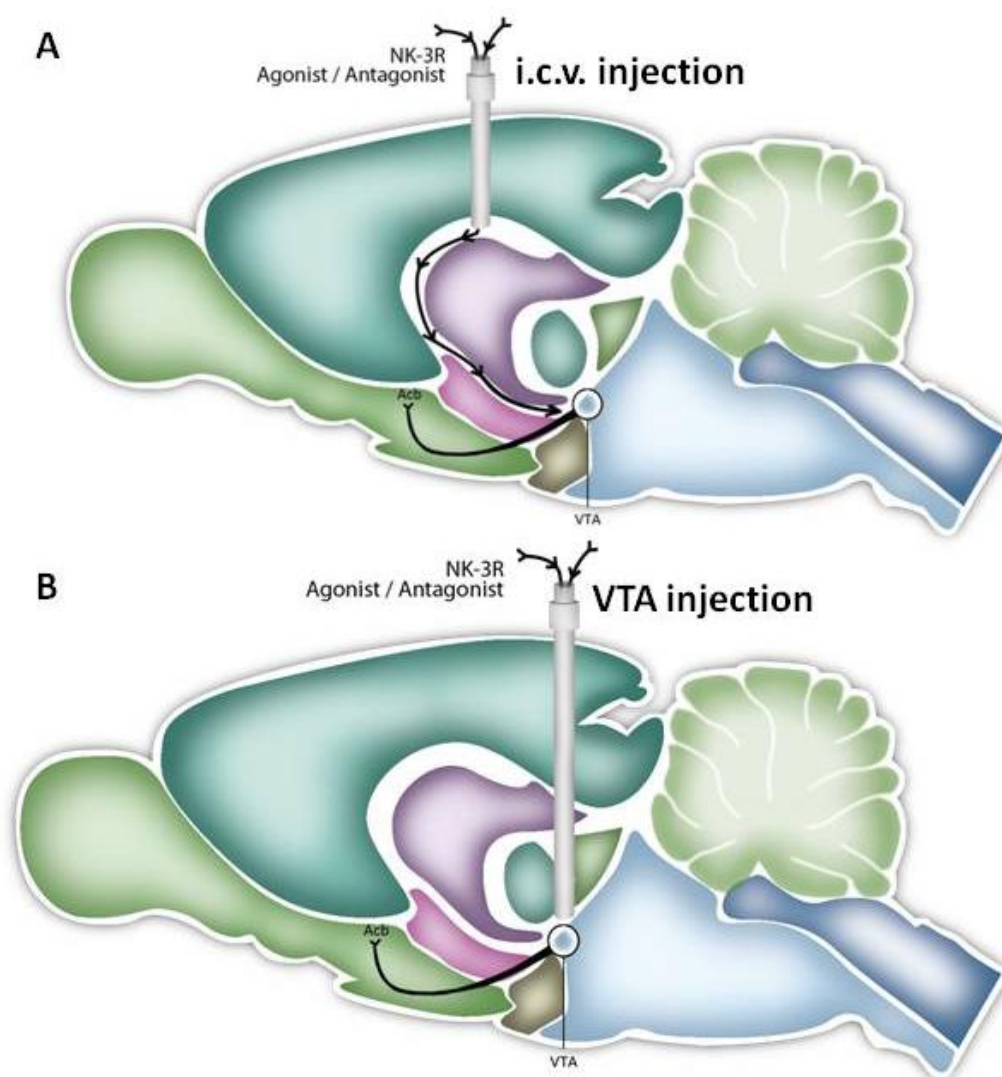


Figure 12. Schematic representation showing tachykinin NK-3R agonist/antagonists injected i.c.v. (A) and VTA (B) in rats.

The cardiovascular response to central injection of senktide in SHR is likely mediated by DA release, since it was abolished after treatment with SCH23390 and haloperidol. The lack of effect of raclopride suggests the involvement of DA-D1R rather than DA-D2R, as reported previously in normotensive rats (Deschamps and Couture, 2005). Blocking the NK-3R led to a reduction of dopamine activity on DA-D2R (Spooren *et al.*, 2005), which is consistent with the prevention of the anti-hypertensive effects of tachykinin NK-3R antagonists (i.c.v. and VTA) by raclopride, the DA-D2R antagonist.

Studies by *in situ* hybridization showed significant higher levels of DA-D2R mRNA in the caudate-putamen, nucleus accumbens, olfactory tubercle, and SN of SHR in comparison with control WKY rats (Vaughan *et al.*, 1999). The increased levels of DA-D2R gene expression displayed in SHR suggest an enhanced central dopaminergic activity in the pathogenesis of hypertension, which might also be partly due to increased tonic activation of the NK-3R making input on this system.

The anti-hypertensive effects of NK-3R antagonists (SB222200 and R-820) injected directly into the VTA were faster in onset than by i.c.v. injection, which is consistent with a site of action in this brain area.

The cardiovascular response caused by senktide in the VTA of SHR was blocked by the prior i.c.v. injection of the NK-3R antagonist R-820, suggesting that i.c.v. injected NK-3R antagonist diffuse to the VTA. Reciprocally, the cardiovascular effect of i.c.v. senktide was prevented by

blockade of NK-3R in the VTA. Moreover, the anti-hypertensive effect of SB222200 injected into the VTA was blocked after i.c.v injection of senktide. Finally, chemical lesion of VTA with ibotenic acid inhibited the cardiovascular effects caused by senktide and R-820 injected i.c.v. Consequently, these findings suggest that the VTA and its brain DA projections are of primary importance in the cardiovascular effects associated with i.c.v. injection of NK-3R agonist and antagonist.

When the NK-3R antagonists (R-820 and SB222200) were injected into the VTA of SHR, a tachycardia was observed. The same dose of antagonists (500 pmol) caused a small bradycardia when injected i.c.v., coinciding with the peak of the anti-hypertensive effect. Hence, the inhibition of NK-3R in the VTA did not interfere with the activation of baroreceptors contrary to i.c.v. injection of NK-3R antagonists. This also suggests that the mechanism of anti-hypertension associated with NK-3R antagonists in the VTA was unlikely due to the inhibition of the cardiac output.

I.c.v. injections of R-820 in SHR for five consecutive days caused a significant decrease in BP accompanied by bradycardia, suggesting that the NK-3R is under constant and chronic tonic activation in SHR. Since the anti-hypertensive effect of NK-3R antagonists was prevented by raclopride, it is concluded that this effect is mainly mediated by DA-D2R. This is consistent with the existence of endogenous tachykinins in the VTA (Kalivas *et al.*, 1985; Warden and Young, 1988; Tamiya *et al.*, 1990; Lu *et al.*, 1998) and the NK-3R on dopaminergic neurons in the VTA (Chen *et al.*, 1998; Lessard *et al.*, 2007) (Figure 13).

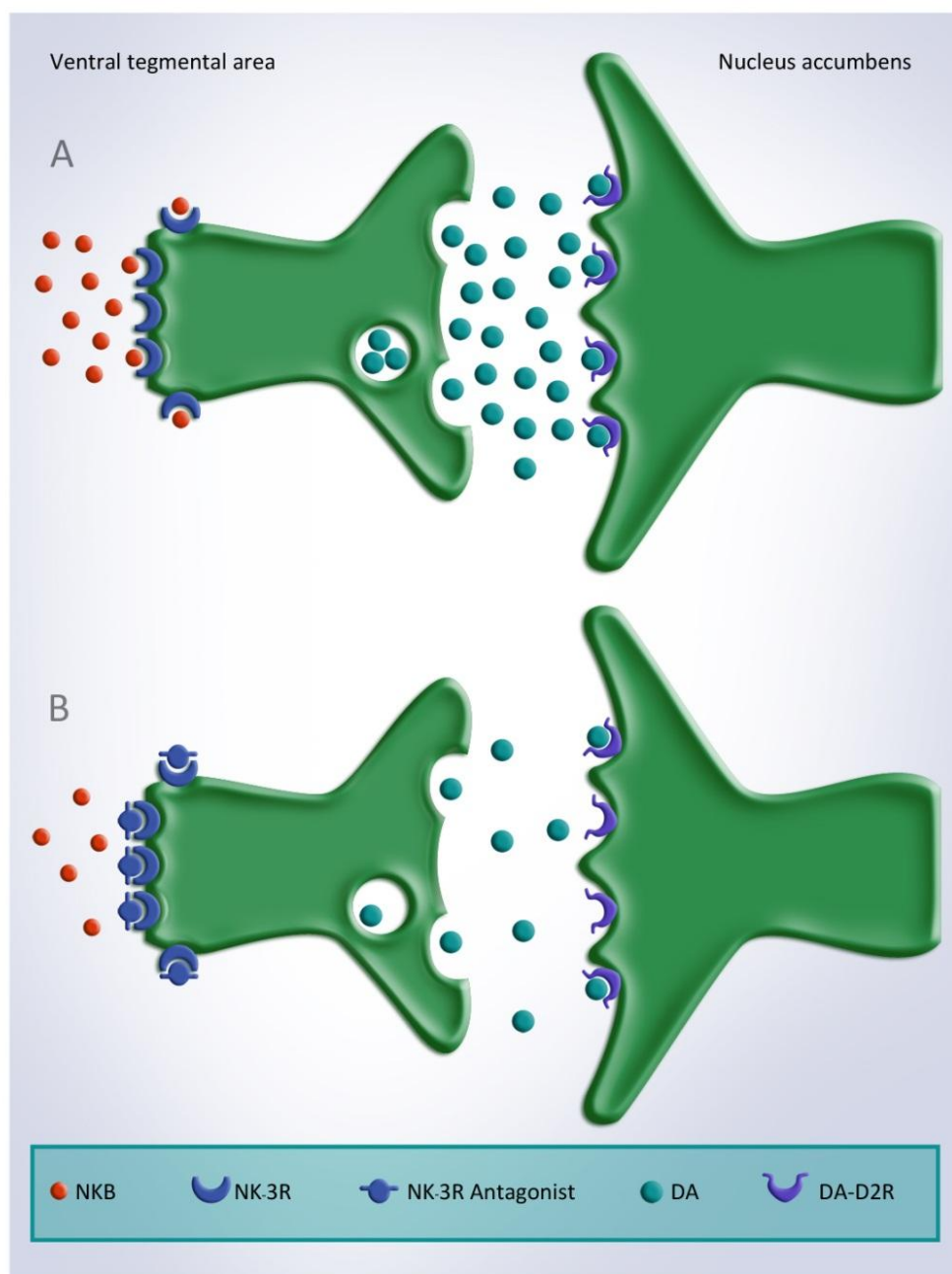


Figure 13. Schematic representation of the NK-3R agonist and antagonists in the CNS. In A, upper panel shows that NKB is released and caused high occupancy of NK-3R. DA neurons are excited by NKB, resulting in an increase of DA release in the nucleus accumbens and a high level of DA-D2R occupancy post-synaptically. In B, lower panel illustrates the inhibition of NKB effect by NK-3R antagonist. As consequence, the DA neurons are less excited and the release of DA in the nucleus accumbens is strikingly decreased. *Adapted from Spooren et al., 2005.*

13.2 Central kinin B1 receptors in hypertension and behavioural activity

The second objective of this thesis was to assess, for the first time, the relative contribution of central B1R in hypertension. The first animal model used was SHR and the second was the rat treated with chronic infusion of Ang II for two weeks, which corresponds to an appropriate experimental model of arterial hypertension associated with increased vascular oxidative stress (Laplane *et al.*, 2003). The B1R antagonist SSR240612 injected i.c.v. produced a significant anti-hypertensive effect in both animal models of hypertension. SSR240612 is expected to spread within the brain through the CSF pathways (Figure 14). In contrast, systemic treatments with R-954 and R-715 caused a small decrease in blood pressure. These later two peptide antagonists do not penetrate into the CNS. SSR240612 crosses the blood-brain barrier and its anti-hypertensive effect produced by systemic or i.c.v. administration was prevented by the DA-D2R antagonists raclopride and haloperidol. Thus, our data suggest that B1R might contribute to the over-activity of the DA system reported in SHR (van den Buuse, 1997). Our results are supported by the increased density of B1R binding sites in the CNS, including the spinal cord (Petcu *et al.*, 2005) and several brain regions (SN, septum, posterodorsal tegmental nucleus and temporal cortex) in Ang II-hypertensive rats (De Brito Pereira *et al.*, 2008). In SHR, autoradiography studies also showed high densities of B1R binding sites in cortical areas, thalamic regions, amygdala and hippocampus, and in some pressor-related hypothalamic areas, such as the

dorsal hypothalamic area and ventromedial hypothalamic nucleus (de Wardener, 2001; Cloutier *et al.*, 2004).

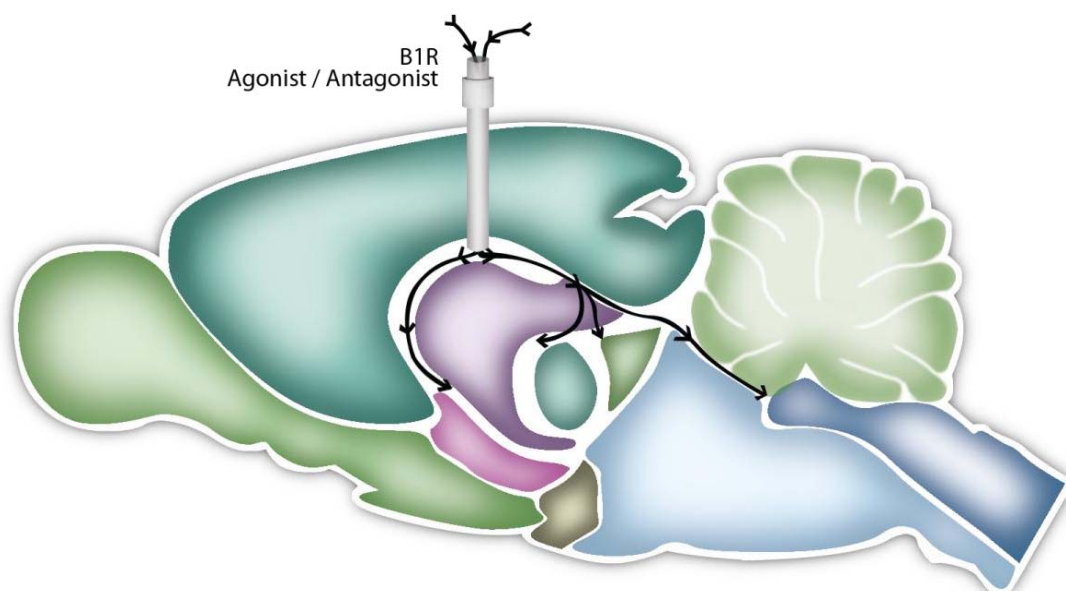


Figure 14. Schematic representation showing the CSF circulation of kinin B1R agonist and antagonists injected i.c.v. in rats.

The inhibition of the oxidative stress with a diet supplemented with alpha-lipoic acid reversed the upregulation of B1R, arterial hypertension and the increased production of vascular superoxide anion, suggesting that B1R is induced by the oxidative stress and contributes to hypertension in the model of Ang II-treated rats (Petcu *et al.*, 2005).

The third objective of this thesis was to assess the contribution of central B1R on behavioural activity. This was to provide further evidence that the increased expression of B1R binding sites in the brain of hypertensive rats corresponds to functional receptors. The objective was not to link hypertension to behavioural activity but to use this paradigm as model of functional B1R activity in the brain. The stimulation of B1R by the agonist Sar[DPhe⁸][des-Arg⁹]BK injected i.c.v. in SHR and Ang II-treated rats enhanced behavioural and locomotor activity through a mechanism involving SP, DA, glutamate and NO. Both hypertensive rat models showed an over-expression of B1R mRNA in the hypothalamus, VTA and nucleus accumbens. However, in control WKY rats, B1R was weakly expressed in the same brain areas and its stimulation did not cause any behavioural effects. These results as well as those obtained with SP and DA antagonists suggest that the mesolimbic dopaminergic system could be a strategic site for the behavioural manifestations induced by the B1R agonist.

Amongst the originalities of this thesis are the demonstration that: 1- similarities occur between NK-3R and B1R in CNS function; and 2- the blockade of either receptor decreases high blood pressure by a central

dopaminergic mechanism. However, future experiments are needed to determine whether the B1R are located on dopamine neurons as already documented for NK-3R (Deschamps and Couture, 2005; Spooren *et al.*, 2005; Lessard *et al.*, 2007; De Brito Gariepy *et al.*, 2010).

B1R are over-expressed in pathological conditions such as diabetes and hypertension (Petcu *et al.*, 2005; Lungu *et al.*, 2007; Dias *et al.*, 2007; present study). It is still unknown, however, whether or not kininase I activity is enhanced. This carboxypeptidase is involved in the formation of des-Arg⁹-BK, the preferred B1R agonist. Studies are therefore needed to measure the activity and the expression of kininase I in the brain of SHR and Ang II-treated rats. Likewise, studies are needed to determine whether BK and its active metabolite des-Arg⁹-BK are increased in specific brain areas of hypertensive rats. This could help our understanding of the anti-hypertensive effect of B1R antagonists. This has to be keeping with the possibility that des-Arg⁹-BK can also upregulate its own B1R (Schanstra *et al.*, 1998). In parallel studies, the endogenous levels of SP and other tachykinins (NKB as a preferred agonist of NK-3R) deserve to be measured to confirm their involvement in hypertension.

Our results on B1R-induced behavioural manifestation highlight the involvement of SP and NO. This is similar to B1R-induced thermal hyperalgesia, which depends on the release of sensory SP and the subsequent production of NO following NK-1R activation in the spinal dorsal horn of type 1 diabetic rats (Couture *et al.*, 2001). One can suggest,

however, that NO is also directly produced after direct activation of B1R through the inducible NO synthase (Ignjatovic *et al.*, 2004; Zhang *et al.*, 2007; Kuhr *et al.*, 2010). The behavioural responses to the B1R agonist were, however, little affected by 1400W, the specific inhibitor of iNOS, suggesting that B1R is not operating primarily via the iNOS pathway.

Interestingly, the profile of NO output generated by B2R-mediated activation of eNOS and B1R-mediated activation of iNOS is extremely different in human lung microvascular endothelial cells (Kuhr *et al.*, 2010). Thus, while B2R activation results in a peak output of NO that lasts for about 5 min, B1R-mediated iNOS activation results in a much more profound and prolonged response, lasting almost 90 min. The specific B1R antagonist, des-Arg¹⁰-Leu⁹-KD, inhibited this response as did the iNOS specific inhibitor 1400W but L-NNA, the eNOS inhibitor, had no effect (Ignjatovic *et al.*, 2004).

According to Zhang *et al.* (2007) iNOS could be acutely activated in a receptor-dependent process. iNOS activity is not regulated by a change in intracellular Ca²⁺ and the phosphorylation sites on eNOS are not conserved in iNOS nor do kinases activating eNOS phosphorylation could activate iNOS (Fulton *et al.*, 1999; Zhang *et al.*, 2007). Further investigation revealed that B1R and iNOS-mediated NO output is dependent on MAPK activation, which results in phosphorylation of iNOS at Ser⁷⁴⁵ (Zhang *et al.*, 2007).

The neuronal NOS (nNOS) produce NO in the central and peripheral nervous system. nNOS also plays a role in cell communication and is associated with plasma membrane. Although, nNOS and eNOS have similar characteristics (Regoli, 2004), the relative contribution of nNOS in behavioural activity induced by brain B1R activation is still unknown. Further studies using specific nNOS inhibitor is compelling to address this issue.

The i.c.v. activation of NK-1R with SP caused characteristic behaviours similar to the defense reaction (Unger *et al.*, 1988; Tschöpe *et al.*, 1992). Those behaviours mimic the behavioural reaction induced by endogenous release of SP in response to morphine withdrawal (Michaud and Couture, 2003) and physiological stress (Culman *et al.*, 1997). Moreover, central SP produced behavioural activities which were blocked by the NK-1R antagonist RP67580 (Picard *et al.*, 1994; Culman *et al.*, 1995; Cellier *et al.*, 1999). The behavioural activity induced by SP was associated with midbrain DA release (Stoessl *et al.*, 1991) which suggests that the release of SP following B1R activation may involve DA neurotransmission and account for the inhibitory effect of D1R and D2R antagonists on centrally mediated behavioural effects of B1R agonist. This is consistent with the participation of DA in the anti-hypertensive effect of B1R antagonist in SHR and Ang II-treated rats (De Brito Gariepy *et al.*, 2010) and with the increased central DA activity described in SHR (Amenta *et al.*, 2001).

Our study focused on the role of the inducible B1R in CNS autonomic and behavioural functions in hypertensive rats. The putative role of brain B2R was not further investigated because it was reported that two-week i.c.v. infusion of B2R antagonist (HOE 140) did not alter systolic BP in comparison to vehicle in SHR (Madeddu *et al.*, 1994). This supports the hypothesis that brain kinin B2R are unlikely involved in the central regulation of blood pressure and hypertension. Nevertheless, B2R may participate in the modulation of baroreceptor reflex sensitivity in SHR (Madeddu *et al.*, 1994).

High concentration of BK in the presence of Ang I-converting enzyme inhibitors (ACEI) and Ang AT2 receptor stimulation (under angiotensin AT1 receptor blockade) provides cardioprotective and nephroprotective effects. Selective kinin B2R agonists also show potential as therapeutic agents in the treatment of several cardiovascular disorders and diabetes mellitus. Ang AT2 receptor activation and Ang (1-7) facilitate the release of BK and NO (via eNOS) in the periphery. Hence, BK in the periphery has a beneficial function by causing vasodilatation, anti-proliferative and anti-fibrotic effects (for review, see Couture and Girolami, 2004).

13.3 Other drugs with central effects

CNS stimulation of α -adrenoceptors leads to bradycardia and a fall in systemic arterial blood pressure. Decrease of blood pressure was induced by i.c.v. administration of L-DOPA in dogs (McCubbin *et al.*, 1960) and with methyldopa in cats and rats (Henning and Van Zwieten, 1968; Day *et*

al., 1972; 1973). In 1972, Heise and Kroneberg showed that the decrease in blood pressure produced by peripheral injection of methyldopa in anaesthetized cats could be prevented by prior treatment with α -adrenoceptor blocking agents. Likewise, the anti-hypertensive effects of clonidine may also be mediated via central α -adrenoceptor stimulation since its action in anesthetized dogs and cats is also inhibited by α -adrenoceptor antagonists (Schmitt and Fenard, 1971). Clonidine has been shown to decrease vasopressin secretion when administered either i.v. or i.c.v. (Reid *et al.*, 1979; Kimura *et al.*, 1981). Inhibition of noradrenaline release following the activation of α -adrenoceptors on noradrenergic sympathetic terminals may also contribute to the anti-hypertensive effect of clonidine (Reid, 1986).

Contrarily to the results with NK-3R and B1R antagonists, CNS stimulation of α -adrenoceptors with clonidine and other agents did not seem to recruit the dopaminergic system. To the best of our knowledge, we do not know any drugs with anti-hypertensive properties which are acting on central dopamine. Most of centrally acting drugs were found to interfere with noradrenergic activity. Clonidine decreases blood pressure by reducing cardiac output and peripheral vascular resistance in addition to causing unwanted depression of the respiratory system (Shen, 2008; Kaczynska and Szereda-Przestaszewska, 2006). This latter side effect of clonidine and those previously described in the Introduction are the main reasons why these drugs were abandoned in the treatment of hypertension. Currently, the anti-hypertensive therapy relies on drugs (calcium antagonists, ACEI, AT1

antagonists, beta and alpha blockers, diuretic) acting chiefly in the periphery (vasculature, kidneys, sympathetic nervous system).

Although the pharmaceutical industry wants to stay away from the CNS in the development of new medication for the treatment of cardiovascular diseases, a better understanding of the mechanism involved in the use of kinin and tachykinin receptor antagonists (NK-3R and B1R) is compelling since these drugs offer great potential for the treatment of psychiatric diseases (schizophrenia and anxiety for NK-3R) and central neuronal diseases (epilepsy, Alzheimer and pain for B1R).

14. Perspectives

The neuropharmacological studies on B1R suggest that this kinin receptor interacts either directly or indirectly with the mesocorticolimbic DA system. Further experiments are needed to confirm the presence of B1R on DA, SP or glutamatergic fibers. This can be addressed by immunocytochemistry using confocal or electron microscopy on the brain of SHR and Ang II-treated rats. Moreover, the anti-hypertensive effect of NK-3R and B1R antagonists are alike, showing a possible cross-talk involving the DA system as the downstream mechanism. Two questions should be addressed: (i) does NK-3R antagonist block the anti-hypertensive effect of B1R antagonist? (ii) does B1R antagonist block the anti-hypertensive effect of NK-3R antagonist? To answer these questions, NK-3R antagonists (R-820 and SB222200) and B1R antagonist (SSR240612) have to be co-administrated i.c.v. in SHR and Ang II-treated rats. If both

receptors interact, no changes in high blood pressure should occur (loss of the anti-hypertensive response). At this time, no evidence suggests a putative interaction at the level of the receptor. It is more likely that NK-3R and B1R increase DA neurotransmission by a redundant or a synergistic mechanism located either on terminals, cells bodies or dendrites of DA neurons.

Several studies have shown in SHR behavioural manifestations (hyperactivity, impulsiveness, poorly sustained attention) similar to the behavioural disturbances occurring in children with attention deficit hyperactivity disorder (ADHD) (for review, see Russell *et al.*, 2005). In SHR, hyperactivity is observed at three to four weeks of age before they enter puberty (De Jong *et al.*, 1995). DOPA decarboxylase activity was found to be enhanced in the midbrain of children and reduced in the prefrontal cortex of adults with ADHD in comparison to controls (Ernst *et al.*, 1998). SHR is also a model exhibiting alterations in the central DA system (Russell *et al.*, 2005). Although the exact mechanism by which altered brain DA affects ADHD is still unknown, SHR may represent a suitable model for studying ADHD. This may or may not relate to B1R, NK-3R or both receptors function. This possibility, however, is supported by my observation that tachykinin NK-3R antagonist injected i.c.v. caused a sedative-type response in SHR and the same treatment with kinin B1R antagonist, SSR240612, decreased the hyper-locomotor activity. Thus, the anxiolytic, anti-depressor or both effects of these drugs deserve to be

examined. The putative role of B1R in anxiety warrants further investigation.

As the VTA is believed to be the site of action of tachykinin NK-3R agonist and antagonist, it would be interesting to investigate the VTA as a putative target for i.c.v. B1R agonists and antagonists. This should also further our understanding of the similar anti-hypertensive effects observed with B1R and NK-3R antagonists.

The next challenge will be to study the induction of B1R linked to the oxidative stress in hypertension. The oxidative stress and the amount of the two pro-oxidative peptides (Ang II and endothelin-1 (ET-1)) increased in hypertension. Also, Ang II and ET-1 enhanced kinin B1R expression in vascular smooth muscle cells (VSMC) (Kintsurashvili *et al.*, 2001; Fernandes *et al.*, 2006; Morand-Contant *et al.*, 2010). As Ang II increased B1R expression through ET-1 release in VSMC, it would be interesting to explore the effect of ET-1 receptor blockade on B1R expression and function in rat models of hypertension (SHR and Ang II-treated rats).

In the same experimental rat models of hypertension, we found that kinin receptor antagonists (SSR240612, R-715 and R-954) given i.c.v. or in the periphery caused a decrease in body temperature for several hours (De Brito Pereira *et al.*, 2008) (Figure 15, unpublished results). However, SSR240612 was inactive in control WKY. This finding suggests that peripheral and central kinin B1R are also involved in the control of core

temperature. Experiments are under way to investigate the mechanism of action of kinin B1R on body temperature.

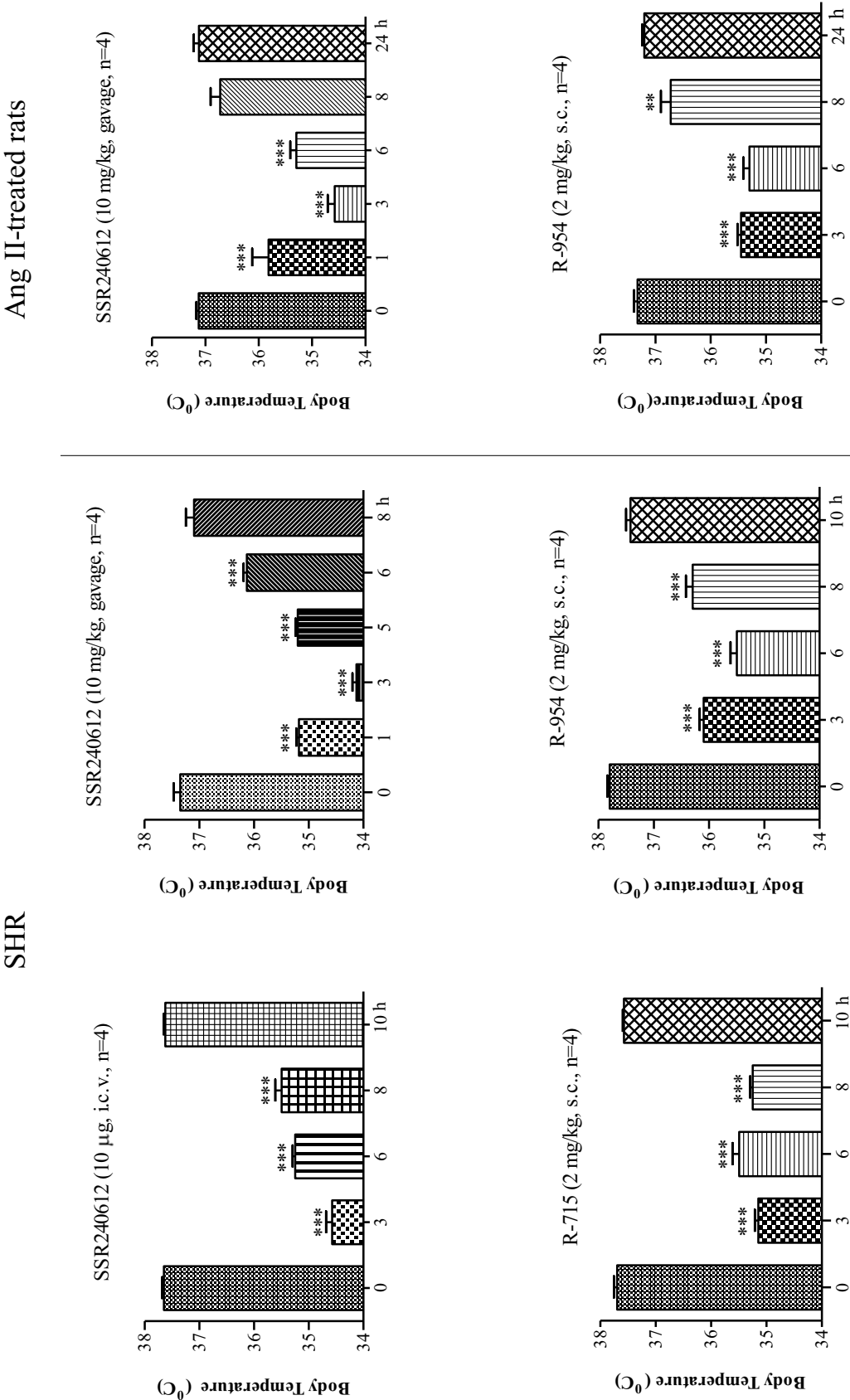


Figure 15 Kinin B1R antagonists (SSR240612, R-954, R-715) injected either by gavage, i.c.v. or s.c. decreased body temperature in SHR and Ang II-treated rats. **P<0,01; ***P<0,001 versus time 0.

15. General conclusion

Salient findings of this thesis are:

- I. Our neuropharmacological studies suggest that the tachykinin NK-3R located in the VTA is submitted to persisting tonic activation which increases midbrain DA transmission and thereby contributes to hypertension in SHR. The VTA may represent a central cardiovascular regulatory site for tachykinin NK-3R in hypertensive rat.
- II. Brain kinin B1R contributes through a central dopaminergic mechanism (DA-D2R) to the maintenance of arterial hypertension in SHR and Ang II-treated rats.
- III. I.c.v. injection of kinin B1R agonist induces stereotypic behaviours and locomotor activity in both animal models of hypertension; SHR and Ang II-treated rats. This was associated with a marked induction of B1R in the hypothalamus, VTA and nucleus accumbens. The B1R-induced behavioural responses are mediated by several central mediators, particularly SP (NK-1R), DA (D1R and D2R), glutamate (NMDA receptor) and nitric oxide.

16. References

- AbdAlla S, Abdel-Baset A, Lothar H., el Massiery A, Quitterer U (2005). Mesangial AT₁/B₂ receptor heterodimers contribute to angiotensin II hyperresponsiveness in experimental hypertension. *J. Mol. Neurosci.* **26**: 185-192.
- Abelous JE, Bardier E (1909). Les substances hypotensives de l'urine humaine normale. *CR Soc Biol.* **66**:511-512.
- Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* **155**(6): 761-767.
- Ackley MA, Asghar AU, Worsley MA, King AE (2001). Peripheral inflammation reduces the response of spinal dorsal horn neurons to an NK-3R agonist. *Neurosci Lett* **308**(1): 13-16.
- Adam A, Albert A, Calay G, Closset J, Damas J, Franchimont P (1985). Human kininogens of low and high molecular mass: quantification by radioimmunoassay and determination of reference values. *Clin Chem.* **31**(3):423-6.
- Advenier C, Naline E, Toty L, Bakdach H, Emonds-Alt X, Vilain P, Brelière JC, Le Fur G. (1992). Effects on the isolated human bronchus of SR 48968, a potent and selective nonpeptide antagonist of the neurokinin A (NK2) receptors. *Am Rev Respir Dis.* **146**:1177-81.
- Almeida e Silva TC, Pela IR (1978). Changes in rectal temperature of the rabbit by intracerebroventricular injection of bradykinin and related kinins. *Agents Actions* **8**(1-2): 102-107.
- Almeida TA, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martin JD (2004). Tachykinins and tachykinin receptors: structure and activity relationships. *Curr Med Chem* **11**(15): 2045-2081.
- Altar CA, Vawter MP, Ginsberg SD (2009). Target identification for CNS diseases by transcriptional profiling. *Neuropsychopharmacology* **34**(1): 18-54.
- Amenta F, Ricci A, Rossodivita I, Avola R, Tayebati SK (2001). The dopaminergic system in hypertension. *Clin Exp Hypertens* **23**(1-2): 15-24.

Amery A, Verstraete M, Bossaert H, Verstreken G (1970). Hypotensive action and side effects of clonidine-chlorthalidone and methyldopa-chlorthalidone in treatment of hypertension. *Br Med J*. **4**:392-5.

Andrade SO, Diniz CR, Rocha e Silva M (1953). Purification of bradykinin by ion exchange chromatography. *Arch. int. Pharmacodyn.* 95-100.

Alric C, Pecher C, Bascands JL, Girolami JP (1999). Effect of bradykinin on tyrosine kinase and phosphatase activities and cell proliferation in mesangial cells. *Immunopharmacology*. **45**(1-3):57-62.

Appenrodt E, Pharow L, Brattstrom A (1993). Effects of central substance P on baroreflex regulation. *Neuropeptides* **25**(1): 77-81.

Arai H, Emson PC (1986). Regional distribution of neuropeptide K and other tachykinins (neurokinin A, neurokinin B and substance P) in rat central nervous system. *Brain Res* **399**(2): 240-249.

Aramori I, Zenkoh J, Morikawa N, O'Donnell N, Asano M, Nakamura K, Iwami M, Kojo H, Notsu Y (1997). Novel subtype-selective nonpeptide bradykinin receptor antagonists FR167344 and FR173657. *Mol. Pharmacol.* **51**: 171-176 (a).

Aramori I, Zenkoh J, Morikawa N, Asano M, Hatori C, Saway H, Kayakiri H, Satoh S, Inoue T, Abe Y, Sawada Y, Mizutani T, Inamura N, Nakahara K, Kojo H, Oku T, Notsu Y (1997). Nonpeptide mimic of bradykinin with long-acting properties at the bradykinin B2 receptor. *Mol. Pharmacol.* **52**:16-20.b

Audet R, Petitclerc E, Drapeau G, Rioux F, Marceau F (1994). Further analysis of the up-regulation of bradykinin B1 receptors in isolated rabbit aorta by using metabolic inhibitors. *Eur J Pharmacol* **271**(2-3): 551-555.

Austin CE, Faussner A, Robinson HE, Chakravarty S, Kyle DJ, Bathon JM, Proud D (1997). Stable expression of the human kinin B1 receptor in Chinese hamster ovary cells. Characterization of ligand binding and effector pathways. *J Biol Chem.* **272**(17):11420-5.

Bachvarov DR, Saint-Jacques E, Larrivee JF, Levesque L, Rioux F, Drapeau G (1995). Cloning and pharmacological characterization of the rabbit bradykinin B2 receptor. *J Pharmacol Exp Ther* **275**(3): 1623-1630.

Bachvarov DR, Hess JF, Menke JG, Larrivée JF, Marceau F (1996). Structure and genomic organization of the human B1 receptor gene for kinins (BDKRB1). *Genomics* **33**(3):374-81.

Bannon MJ, Elliott PJ, Alpert JE, Goedert M, Iversen SD, Iversen LL (1983). Role of endogenous substance P in stress-induced activation of mesocortical dopamine neurones. *Nature* **306**(5945): 791-792.

Bannon MJ, Deutch AY, Tam SY, Zamir N, Eskay RL, Lee JM, Maggio JE, Roth RH (1986). Mild footshock stress dissociates substance P from substance K and dynorphin from Met- and Leu-enkephalin. *Brain Res* **381**(2):393-396.

Bannon MJ, Poosch MS, Haverstick DM, Mandal A, Xue IC, Shibata K (1992). Preprotachykinin gene expression in the human basal ganglia: characterization of mRNAs and pre-mRNAs produced by alternate RNA splicing. *Brain Res Mol Brain Res* **12**(1-3): 225-231.

Barnes JM, Barnes NM, Costall B, Cox AJ, Domeney AM, Kelly ME (1990). Neurochemical consequences following injection of the substance P analogue, DiMe-C7, into the rat ventral tegmental area. *Pharmacol Biochem Behav* **37**(4): 839-841.

Barnes PJ (1998). Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* **94**(6): 557-572.

Bascands JL, Marin Castaño ME, Bompard G, Pecher C, Gaucher M, Girolami JP (1996). Postnatal maturation of the Kallikrein-kinin system in the rat kidney: from enzyme activity to receptor gene expression. *J Am Soc Nephrol* **7**(1):81-9.

Bascands JL, Schanstra JP, Couture R, Girolami JP (2003). [Bradykinin receptors: towards new pathophysiological roles]. *Med Sci (Paris)*. **19**(11):1093-100.

Barr AJ, Watson SP (1994). Protein kinase C mediates delayed inhibitory feedback regulation of human neurokinin type 1 receptor activation of phospholipase C in UC11 astrocytoma cells. *Mol Pharmacol* **46**(2): 266-273.

Barton BE, Jackson JV. Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. *Infect Immun* **61**(4):1496-1499.

Beaujouan JC, Saffroy M, Torrens Y, Glowinski J (2000). Different subtypes of tachykinin NK(1) receptor binding sites are present in the rat brain. *J Neurochem* **75**(3): 1015-1026.

Beaujouan JC, Saffroy M, Torrens Y, Glowinski J (1997). Potency and selectivity of the tachykinin NK-3R antagonist SR 142801. *Eur J Pharmacol* **319**(2-3): 307-316.

Benarroch EE (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin Proc* **68**(10): 988-1001.

Benetato G, Haulica I, Muscalu I, Bubuianu E, Galesanu S (1964). On the central nervous action of bradykinin. *Rev Rouman Physiol* **1**:313-318.

Bennett VJ, Simmons MA (2001). Analysis of fluorescently labeled substance P analogs: binding, imaging and receptor activation. *BMC Chem Biol* **1**(1): 1.

Beresford IJ, Sheldrick RL, Ball DI, Turpin MP, Walsh DM, Hawcock AB, Coleman RA, Hagan RM, Tyers MB (1995). GR159897, a potent non-peptide antagonist at tachykinin NK2 receptors. *Eur J Pharmacol* **272**(2-3):241-8.

Bergevin A, Girardot D, Bourque MJ, Trudeau LE (2002). Presynaptic mu-opioid receptors regulate a late step of the secretory process in rat ventral tegmental area GABAergic neurons. *Neuropharmacology* **42**(8): 1065-1078.

Bhoola KD (1996). Translocation of the neutrophil kinin moiety and changes in the regulation of kinin receptors in inflammation. *Immunopharmacology* **33**(1-3): 247-256.

Bhoola KD, Elson CJ, Dieppe PA (1992). Kinins--key mediators in inflammatory arthritis? *Br J Rheumatol* **31**(8): 509-518.

Blaukat A, Dikic I (2001). Activation of sphingosine kinase by the bradykinin B2 receptor and its implication in regulation of the ERK/MAP kinase pathway. *Biol Chem* **382**(1):135-139.

Bock C, Niederhoffer N, Szabo B (1999). Analysis of the receptor involved in the central hypotensive effect of rilmenidine and moxonidine. *Naunyn Schmiedebergs Arch Pharmacol* **359**(4):262-271.

Blount P, Krause JE (1993). Functional nonequivalence of structurally homologous domains of neurokinin-1 and neurokinin-2 type tachykinin receptors. *J Biol Chem*. 268(22):16388-95.

Bouron A, Reuter H (1999). The D1 dopamine receptor agonist SKF-38393 stimulates the release of glutamate in the hippocampus. *Neuroscience* **94**:1063-1070.

Bouthenet ML, Martres MP, Sales N, Schwartz JC (1987). A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [125I]iodosulpride. *Neuroscience* **20**(1): 117-155.

Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC (1991). Localization of dopamine D3 receptor mRNA in the rat brain using in situ hybridization histochemistry: comparison with dopamine D2 receptor mRNA. *Brain Res* **564**(2): 203-219.

Bregola G, Varani K, Gessi S, Beani L, Bianchi C, Borea PA, Regoli D, Simonato M (1999). Changes in hippocampal and cortical B1 bradykinin receptor biological activity in two experimental models of epilepsy. *Neuroscience* **92**(3):1043-1049.

Bricca G, Dontenwill M, Molines A, Feldman J, Belcourt A, Bousquet P (1989). The imidazoline preferring receptor: binding studies in bovine, rat and human brainstem. *Eur J Pharmacol* **162**(1):1-9.

Brody MJ, O'Neil TP, Porter JP (1984). Role of central catecholaminergic systems in the pathogenesis and treatment of hypertension. *J Cardiovasc Pharmacol* **6**: S727-S741.

Brownstein MJ, Mroz EA, Kizer JS, Palkovits M, Leeman SE (1976). Regional distribution of substance P in the brain of the rat. *Brain Res* **116**(2):299-305.

Buck SH, Helke CJ, Burcher E, Shults CW, O'Donohue TL (1986). Pharmacologic characterization and autoradiographic distribution of binding sites for iodinated tachykinins in the rat central nervous system. *Peptides* **7**(6): 1109-1120.

Bunag RD, Takahashi H (1981). Exaggerated sympathetic responses to bradykinin in spontaneously hypertensive rats. *Hypertension* **3**: 433-440.

Cabrini DA, Campos MM, Tratsk KS, Merino VF, Silva JA, Jr., Souza GE (2001). Molecular and pharmacological evidence for modulation of kinin B(1) receptor expression by endogenous glucocorticoids hormones in rats. *Br J Pharmacol* **132**(2): 567-577.

Cador M, Rivet JM, Kelley AE, Le Moal M, Stinus L (1989). Substance P, neurotensin and enkephalin injections into the ventral tegmental area: comparative study on dopamine turnover in several forebrain structures. *Brain Res* **486**(2): 357-363.

Caldeira JC, Franci CR, Pelá IR (1998). Bilateral lesion of hypothalamic paraventricular nucleus abolishes fever induced by endotoxin and bradykinin in rats. *Ann N Y Acad Sci* **856**:294-297.

Calixto JB, Cabrini DA, Ferreira J, Campos MM (2001). Inflammatory pain: kinins and antagonists. *Curr Opin Anaesthesiol* **14**(5):519-526.

Calixto JB, Cabrini DA, Ferreira J, Campos MM (2000). Kinins in pain and inflammation. *Pain* **87**(1): 1-5.

Camargo AC, Ramalho-Pinto FJ, Greene LJ (1972). Brain peptidases: conversion and inactivation of kinin hormones. *J Neurochem* **19**(1):37-49.

Camargo AC, Shapanka R, Greene LJ (1973). Preparation, assay, and partial characterization of a neutral endopeptidase from rabbit brain. *Biochemistry* **12**: 1838-1844.

Campos AH, Calixto JB (1994). Mechanisms involved in the contractile responses of kinins in rat portal vein rings: mediation by B1 and B2 receptors. *J Pharmacol Exp Ther* **268**(2): 902-909.

Campos MM, Henriques MG, Calixto JB (1997). The role of B1 and B2 kinin receptors in oedema formation after long-term treatment with *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). *Br J Pharmacol* **120**(3): 502-508.

Campos MM, Mata LV, Calixto JB (1995). Expression of B1 kinin receptors mediating paw edema and formalin-induced nociception. Modulation by glucocorticoids. *Can J Physiol Pharmacol* **73**(7): 812-819.

Campos MM, Ongali B, De Souza Buck H, Schanstra JP, Girolami JP, Chabot JG (2005). Expression and distribution of kinin B1 receptor in the rat brain and alterations induced by diabetes in the model of streptozotocin. *Synapse* **57**(1): 29-37.

Campos MM, Souza GE, Calixto JB (1999). In vivo B1 kinin-receptor up-regulation. Evidence for involvement of protein kinases and nuclear factor kappaB pathways. *Br J Pharmacol* **127**(8): 1851-1859.

Campos MM, Souza GE, Calixto JB (1998). Modulation of kinin B1 but not B2 receptors-mediated rat paw edema by IL-1beta and TNFalpha. *Peptides* **19**(7): 1269-1276.

Campos MM, Leal PC, Yunes RA, Calixto JB (2006). Non-peptide antagonists for kinin B1 receptors: new insights into their therapeutic potential for the management of inflammation and pain. *Trends Pharmacol Sci* **27**(12):646-651.

Cao BJ, Rodgers RJ (1998). Tolerance to acute anxiolysis but no withdrawal anxiogenesis in mice treated chronically with 5-HT1A receptor antagonist, WAY 100635. *Neurosci Biobehav Rev* **23**(2): 247-257.

Capriles N, Rodaros D, Sorge RE, Stewart J (2003). A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)* **168**(1-2): 66-74.

Catalioto RM, Cucchi P, Renzetti AR, Criscuoli M, Maggi CA (1998). Independent coupling of the human tachykinin NK2 receptor to phospholipases C and A2 in transfected Chinese hamster ovary cells. *Naunyn Schmiedeberg Arch Pharmacol*. **358**(4):395-403.

Carlsson A, Lindqvist M (1963). Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. *Acta Pharmacol Toxicol (Copenh)* **20**:140-144.

Carr DB, Sesack SR (2000). Dopamine terminals synapse on callosal projection neurons in the rat prefrontal cortex. *J Comp Neurol* **425**(2): 275-283.

Cellier E, Barbot L, Iyengar S, Couture R (1999). Characterization of central and peripheral effects of septide with the use of five tachykinin NK-1 receptor antagonists in the rat. *Br J Pharmacol* **127**(3): 717-728.

Cellier E, Barbot L, Regoli D, Couture R (1997). Cardiovascular and behavioural effects of intracerebroventricularly administered tachykinin NK-3R antagonists in the conscious rat. *Br J Pharmacol* **122**(4): 643-654.

Ceravolo GS, Fernandes L, Munhoz CD, Fernandes DC, Tostes RC, Laurindo FR (2007). Angiotensin II chronic infusion induces B1 receptor expression in aorta of rats. *Hypertension* **50**(4): 756-761.

Chahl LA (2006). Tachykinins and neuropsychiatric disorders. *Curr Drug Targets* **7**(8): 993-1003.

Chai KX, Ni A, Wang D, Ward DC, Chao J, Chao L (1996). Genomic DNA sequence, expression and chromosomal localization of the human B1 bradykinin receptor gene BDKRB1. *Genomics* **31**:51-57.

Chan-Palay V (1978). Autoradiographic localization of gamma-aminobutyric acid receptors in the rat central nervous system by using [³H]muscimol. *Proc Natl Acad Sci USA* **75**(2): 1024-1028.

Chan JY, Barnes CD, Chan SH (1990). Tonic enhancement of the sensitivity of baroreceptor reflex response by endogenous substance P in the rat. *Regul Pept* **29**(2-3): 199-213.

Chan OH, Schmid HL, Kuo BS, Wright DS, Howson W, Stewart BH (1996). Absorption of Cam-2445, and NK-1 neurokinin receptor antagonist: in vivo, in situ, and in vitro evaluations. *J Pharm Sci* **85**(3): 253-257.

Chang MM, Leeman SE (1970). Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J Biol Chem* **245**(18): 4784-4790.

Charlton CG, Helke CJ (1987). Substance P-containing medullary projections to the intermediolateral cell column: identification with retrogradely transported rhodamine-labeled latex microspheres and immunohistochemistry. *Brain Res* **418**(2):245-54.

Chao J, Chao L (1987). Identification and expression of kallikrein gene family in rat submandibular and prostate glands using monoclonal antibodies as specific probes. *Biochim Biophys Acta* **910**(3): 233-239.

Chassaing G, Lavielle S, Loeuillet D, Robillard P, Carruette A, Garret C, Beaujouan JC, Saffroy M, Petitet F, Torrens Y, Glowinski J (1991). Selective agonists of NK-2 binding sites highly active on rat portal vein (NK-3 bioassay). *Neuropeptides* **19**: 91-95.

Chapman and Hall (1993). Dopamine. In: Neurotransmitters and drugs, 3rd edition. Kruk ZL and Pycock CJ. London, England : 87-115.

Chavolla-Calderon M, Bayer MK, Fontan JJ (2003). Bone marrow transplantation reveals an essential synergy between neuronal and hemopoietic cell neurokinin production in pulmonary inflammation. *J Clin Invest* **111**(7): 973-980.

Chen LW, Guan ZL, Ding YQ (1998). Mesencephalic dopaminergic neurons expressing neuromedin K receptor (NK3): a double immunocytochemical study in the rat. *Brain Res* **780**: 150-154.

Chen NN, Pan WH (2000). Regulatory effects of D2 receptors in the ventral tegmental area on the mesocorticolimbic dopaminergic pathway. *J Neurochem* **74**(6): 2576-2582.

Chen EY, Emerich DF, Bartus RT, Kordower JH (2000). B2 bradykinin receptor immunoreactivity in rat brain. *J Comp Neurol* **427**(1):1-18.

Churchill L, Dilts RP, Kalivas PW (1992). Autoradiographic localization of gamma-aminobutyric acidA receptors within the ventral tegmental area. *Neurochem Res* **17**(1): 101-106.

Cloutier F, de Sousa Buck H, Ongali B, Couture R (2002). Pharmacologic and autoradiographic evidence for an up-regulation of kinin B(2) receptors in the spinal cord of spontaneously hypertensive rats. *Br J Pharmacol* **135**(7): 1641-1654.

Cloutier F, Ongali B, Campos MM, Thibault G, Neugebauer W, Couture R (2004). Correlation between brain bradykinin receptor binding sites and cardiovascular function in young and adult spontaneously hypertensive rats. *Br J Pharmacol* **142**(2): 285-296.

Cloutier F, Ongali B, Deschamps K, Brouillette J, Neugebauer W, Couture R (2006). Up-regulation of tachykinin NK-1 and NK-3 receptor binding sites in the spinal cord of spontaneously hypertensive rat: impact on the autonomic control of blood pressure. *Br J Pharmacol* **148**(1): 25-38.

Coelho MM, Oliveira CR, Pajolla GP, Calixto JB, Pela IR (1997). Central involvement of kinin B1 and B2 receptors in the febrile response induced by endotoxin in rats. *Br J Pharmacol* **121**(2): 296-302.

Cole TJ, Schreiber G (1992). The structure and expression of the genes for T-kininogen in the rat. *Agents Actions Suppl* **38**: 292-299.

Contreras F, Fouillieux C, Bolivar A, Simonovis N, Hernandez-Hernandez R, Armas-Hernandez MJ (2002). Dopamine, hypertension and obesity. *J Hum Hypertens* **16**: S13-17.

Copeland LA, Zeber JE, Rosenheck RA, Miller AL (2006). Unforeseen inpatient mortality among veterans with schizophrenia. *Med Care* **44**(2): 110-116.

Cornish JL, van den Buuse M (1994). Pressor responses to electrical and chemical stimulation of the rat brain A10 dopaminergic system. *Neurosci Lett* **176**(2): 142-146.

Cornish JL, van den Buuse M (1995). Stimulation of the rat mesolimbic dopaminergic system produces a pressor response which is mediated by dopamine D-1 and D-2 receptor activation and the release of vasopressin. *Brain Res* **701**(1-2):28-38.

Cornish JL, Wilks DP, van den Buuse M (1997) A functional interaction between the mesolimbic dopamine system and vasopressin release in the regulation of blood pressure in conscious rats. *Neuroscience* **81**:69–78.

Corrêa FM, Graeff FG (1974). Central mechanisms of the hypertensive action of intraventricular bradykinin in the unanaesthetized rat. *Neuropharmacology* **13**(1): 65-75.

Corrêa FM, Graeff FG (1976). On the mechanism of the hypertensive action of intraseptal bradykinin in the rat. *Neuropharmacology* **15**(11):713-717.

Corrêa FM, Innis RB, Uhl GR, Snyder SH (1979). Bradykinin-like immunoreactive neuronal systems localized histochemically in rat brain. *Proc Natl Acad Sci U S A* **76**(3): 1489-1493.

Coskran, TM, Morton, DG, Menniti, FS, Adamowicz, WO, Kleiman, RJ, Ryan, AM, Strick, CA, Schmidt, CJ and Stephenson, DT (2006) Immunohistochemical localization of phosphodiesterase 10A, PDE10A, in multiple mammalian species. *J. Histochem. Cytochem* **54**:1205-1213.

Costa R, Fernandes ES, Menezes-De-Lima OJR, Campos MM, Calixto JB (2006). Effect of novel selective non-peptide kinin B1 receptor antagonists on mouse pleurisy induced by carrageenan. *Peptides* **27**:2967–2975.

Couture R, Mizrahi J, Regoli D, Devroede G (1981). Peptides and the human colon: an in vitro pharmacological study. *Can J Physiol Pharmacol* **59**(9): 957-964.

Couture R, Picard P, Poulat P, Prat A (1995). Characterization of the tachykinin receptors involved in spinal and supraspinal cardiovascular regulation. *Can J Physiol Pharmacol* **73**(7): 892-902.

Couture R, Toma N, Barbot L (2000). SR142801 behaves as a tachykinin NK-3 receptor agonist on a spinal nociceptive reflex in the rat. *Life Sci* **66**(1): 51-65.

Couture R and Lindsey CJ (2000). Brain Kallikrein-Kinin System: from Receptors to Neuronal Pathways and Physiological Functions. In: Handbook of Chemical Neuroanatomy: Peptide Receptors Part I (Quirion

Björklund RA, Hökfelt T. *Elsevier Science Ltd Amsterdam* Chapter 7 **16**: 241-300.

Couture R, Harrisson M, Vianna RM, Cloutier F (2001). Kinin receptors in pain and inflammation. *Eur J Pharmacol* **429**(1-3): 161-176.

Couture R, Girolami JP (2004). Putative roles of kinin receptors in the therapeutic effects of angiotensin 1-converting enzyme inhibitors in diabetes mellitus. *Eur J Pharmacol* **500**(1-3): 467-485.

Creese I, Morrow AL, Leff SE, Sibley DR, Hamblin MW (1982). Dopamine receptors in the central nervous system. *Int Rev Neurobiol* **23**: 255-301.

Cholewinski AJ, Stevens G, McDermott AM, Wilkin GP (1991). Identification of B2 bradykinin binding sites on cultured cortical astrocytes. *J Neurochem* **57**(4):1456-1458.

Chrisp P, Faulds D (1992). Moxonidine: a review of its pharmacology, and therapeutic use in essential hypertension. *Drugs* **44**: 993-1012.

Christopher J, Jaffa AA (2002). Diabetes modulates the expression of glomerular kinin receptors. *Int Immunopharmacol* **2**(13-14):1771-1779.

Cuello AC, Emson PC, Paxinos G, Jessell T (1978). Substance P containing and cholinergic projections from the habenula. *Brain Res* **149**(2): 413-429.

Culman J, Wiegand B, Spitznagel H, Klee S, Unger T (1995). Effects of the tachykinin NK-1 receptor antagonist, RP 67580, on central cardiovascular and behavioural effects of substance P, neurokinin A and neurokinin B. *Br J Pharmacol* **114**(6): 1310-1316.

Culman J, Unger T (1995). Central tachykinins: mediators of defence reaction and stress reactions. *Can J Physiol Pharmacol* **73**:885-891.

Culman J, Klee S, Ohlendorf C, Unger T (1997). Effect of tachykinin receptor inhibition in the brain on cardiovascular and behavioural responses to stress. *J Pharmacol Exp Ther* **280**(1): 238-246.

Culman J, Das G, Ohlendorf C, Haass M, Maser-Gluth C, Zuhayra M, Zhao Y, Itoi K (2010). Blockade of tachykinin NK(1)/NK(2) receptors in the brain attenuates the activation of CRH-neurons in the hypothalamic

paraventricular nucleus and the sympathoadrenal and pituitary-adrenal responses to formalin-induced pain in the rat. *J Neuroendocrinol* Feb 20 [Epub ahead of print].

Cushman DW, Cheung HS (1971). Concentrations of angiotensin-converting enzyme in tissues of the rat. *Biochim Biophys Acta* **250**(1): 261-265.

Damas J, Delree P, Bourdon V (1992). Distribution of immunoreactive T-kininogen in rat nervous tissues. *Brain Res* **569**(1): 63-70.

Davis AJ, Perkins MN (1994). Induction of B1 receptors in vivo in a model of persistent inflammatory mechanical hyperalgesia in the rat. *Neuropharmacology* **33**(1): 127-133.

Dawson LA, Cato KJ, Scott C, Watson JM, Wood MD, Foxton R (2008). In vitro and in vivo characterization of the non-peptide NK-3R antagonist SB-223412 (talnetant): potential therapeutic utility in the treatment of schizophrenia. *Neuropsychopharmacology* **33**(7): 1642-1652.

Dawson LA, Langmead CJ, Dada A, Watson JM, Wu Z, de la Flor R (2010). In vitro and in vivo comparison of two non-peptide tachykinin NK-3R antagonists: Improvements in efficacy achieved through enhanced brain penetration or altered pharmacological characteristics. *Eur J Pharmacol* **627**(1-3): 106-114.

Dawson TM, Gehlert DR, Wamsley JK (1986). Quantitative autoradiographic localization of central dopamine D-1 and D-2 receptors. *Adv Exp Med Biol* **204**: 93-118.

Day MD, Roach AG, Whiting RL (1972). The mode of action of -methyldopa. *Br J Pharmacol* **45**(1):168P-169P.

Day MD, Roach AG, Whiting RL (1973). The mechanism of the antihypertensive action of -methyldopa in hypertensive rats. *Eur J Pharmacol* **21**(3):271-280.

De Brito Pereira H, Talbot S, Ongali B, Couture R (2008). Role of kinin B1 receptor in the central autonomic control of blood pressure and body temperature in two rat models of hypertension. In: 39th Annual Meeting of

the Society for Neuroscience, November 12–15, Washington, DC, USA (P83.18).

De Brito Gariepy H, Carayon P, Ferrari B, Couture R (2010). Contribution of the central dopaminergic system in the anti-hypertensive effect of kinin B1 receptor antagonists in two rat models of hypertension. *Neuropeptides* **44**(2):191-8.

de Champlain J (2001). Do most antihypertensive agents have a sympatholytic action? *Curr Hypertens Rep* **3**(4): 305-313.

de Champlain J, Karas M, Nguyen P, Cartier P, Wistaff R, Toal CB (1998). Different effects of nifedipine and amlodipine on circulating catecholamine levels in essential hypertensive patients. *J Hypertens* **16**(9): 1357-1369.

de Sousa Buck H, Ongali B, Thibault G, Lindsey CJ, Couture R (2002). Autoradiographic detection of kinin receptors in the human medulla of control, hypertensive, and diabetic donors. *Can J Physiol Pharmacol* **80**(4): 249-257.

de Wardener HE (2001). The hypothalamus and hypertension. *Physiol Rev* **81**(4): 1599-1658.

Deblois D, Bouthillier J, Marceau F (1988). Effect of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to des-Arg9-bradykinin. *Br J Pharmacol* **93**(4): 969-977.

deBlois D, Horlick RA (2001). Endotoxin sensitization to kinin B(1) receptor agonist in a non-human primate model: haemodynamic and pro-inflammatory effects. *Br J Pharmacol* **132**(1): 327-335.

Decarie A, Raymond P, Gervais N, Couture R, Adam A (1996). Serum interspecies differences in metabolic pathways of bradykinin and [des-Arg9]BK: influence of enalaprilat. *Am J Physiol* **271**(4 Pt 2): H1340-1347.

Defagot MC, Antonelli MC (1997). Autoradiographic localization of the putative D4 dopamine receptor in rat brain. *Neurochem Res* **22**(4): 401-407.

De Felipe C, Herrero JF, O'Brien JA, Palmer JA, Doyle CA, Smith AJ, Laird JM, Belmonte C, Cervero F, Hunt SP (1998). Altered nociception, analgesia

and aggression in mice lacking the receptor for substance P. *Nature* **392**(6674):394-7.

De Jong W, Linthorst AC, Versteeg HG (1995). The nigrostriatal dopamine system and the development of hypertension in the spontaneously hypertensive rat. *Arch Mal Coeur Vaiss* **88**:1193-1196.

Del Fiacco M, Levanti MC, Dessi ML, Zucca G (1987). The human hippocampal formation and parahippocampal gyrus: localization of substance P-like immunoreactivity in newborn and adult post-mortem tissue. *Neuroscience* **21**(1):141-50.

Dendorfer A, Wagemann M, Reissmann S, Dominiak P (1999). Structural requirements for B2-agonists with improved degradation stability. *Immunopharmacology* **45**(1-3): 199-205.

Dengler R, Faussner A, Muller-Esterl W, Roscher AA (1990). [Hyp3]-bradykinin and [Hyp3]-Lys-bradykinin interact with B2-bradykinin receptors and stimulate inositol phosphate production in cultured human fibroblasts. *FEBS Lett* **262**(1): 111-114.

Deschamps K, Couture R (2005). The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br J Pharmacol* **145**(6): 712-727.

Deutch AY, Maggio JE, Bannon MJ, Kalivas PW, Tam SY, Goldstein M (1985). Substance K and substance P differentially modulate mesolimbic and mesocortical systems. *Peptides* **6 Suppl 2**: 113-122.

Devillier P, Renoux M, Drapeau G, Regoli D (1988). Histamine release from rat peritoneal mast cells by kinin antagonists. *Eur J Pharmacol* **149**(1-2): 137-140.

Dias JP, Ismael MA, Pilon M, de Champlain J, Ferrari B, Carayon P (2007). The kinin B1 receptor antagonist SSR240612 reverses tactile and cold allodynia in an experimental rat model of insulin resistance. *Br J Pharmacol* **152**(2): 280-287.

Diz DI (1985). Bradykinin and related peptides in central control of the cardiovascular system. *Peptides* **6 Suppl 2**: 57-64.

Diz DI, Jacobowitz DM (1984). Cardiovascular effects produced by injections of thyrotropin-releasing hormone in specific preoptic and hypothalamic nuclei in the rat. *Peptides* **5**(4): 801-808.

Dixon BS (1994) Cyclic AMP selectively enhances bradykinin receptor synthesis and expression in cultured arterial smooth muscle: Inhibition of angiotensin II and vasopressin response. *J Clin Invest* **93**:2535-2544.

Doherty MD, Pickel VM (2000). Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res* **864**(2): 176-185.

Donaldson LF, Hanley MR, Villabranca AC (1997). Inducible receptors. *Trends Pharmacol. Sci* **18**:171-181.

Douglas FL, Palkovits M, Brownstein MJ (1982). Regional distribution of substance P-like immunoreactivity in the lower brainstem of the rat. *Brain Res* **245**(2): 376-378.

Draganic DM, Catts SV, Carr VJ (2007). Neuroscience Institute of Schizophrenia and Allied Disorders (NISAD): 10 years of Australia's first virtual research institute. *Aust N Z J Psychiatry* **41**(1): 78-88.

Drapeau G, D'Orleans-Juste P, Dion S, Rhaleb NE, Rouissi NE, Regoli D (1987). Selective agonists for substance P and neurokinin receptors. *Neuropeptides* **10**:43-54.

Drapeau G, deBlois D, Marceau F (1991). Hypotensive effects of Lys-des-Arg⁹-bradykinin and metabolically protected agonists of B1 receptors for kinins. *J Pharmacol Exp Ther* **259**(3): 997-1003.

Drapeau G, Rouissi N, Nantel F, Rhaleb NE, Tousignant C, Regoli D (1990). Antagonists for the neurokinin NK-3 receptor evaluated in selective receptor systems. *Regul Pept* **31**(2):125-135.

Dray A (1997). Kinins and their receptors in hyperalgesia. *Can J Physiol Pharmacol* **75**(6): 704-712.

Dubois A, Savasta M, Curet O, Scatton B (1986). Autoradiographic distribution of the D1 agonist [3H]SKF 38393, in the rat brain and spinal

cord. Comparison with the distribution of D2 dopamine receptors. *Neuroscience* **19**(1): 125-137.

Dulinski R, Suder P, Guevara-Lora I, Rapala-Kozik M, Potempa J, Silberring J (2003). Attenuated kinin release from human neutrophil elastase-pretreated kininogens by tissue and plasma kallikreins. *Biol Chem* **384**(6): 929-937.

Ebner K, Singewald N (2006). The role of substance P in stress and anxiety responses. *Amino Acids* **31**(3): 251-272.

Eison AS, Eison MS, Iversen SD (1982). The behavioural effects of a novel substance P analogue following infusion into the ventral tegmental area or SN of rat brain. *Brain Res* **238**(1): 137-152.

El Midaoui A, Ongali B, Petcu M, Rodi D, de Champlain J, Neugebauer W (2005). Increases of spinal kinin receptor binding sites in two rat models of insulin resistance. *Peptides* **26**(8): 1323-1330.

Elliott PJ, Iversen SD (1986). Behavioural effects of tachykinins and related peptides. *Brain Res* **381**(1):68-76.

Elliott PJ, Nemeroff CB, Kilts CD (1986b). Evidence for a tonic facilitatory influence of substance P on dopamine release in the nucleus accumbens. *Brain Res* **385**(2): 379-382.

Elliott PJ, Mason GS, Graham EA, Turpin MP, Hagan RM (1992). Modulation of the rat mesolimbic dopamine pathway by neurokinins. *Behav Brain Res* **51**(1): 77-82.

Elliott PJ, Mason GS, Stephens-Smith M, Hagan RM (1991). Behavioural and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. *Neuropeptides* **19**(2): 119-126.

Elrod K, Okamoto H, Greenbaum LM, Buccafusco JJ (1986). Inactivation of kallikrein and kininases and stabilization of whole rat brain kinin levels following focused microwave irradiation. *Neurochem Res* **11**(10): 1463-1471.

Emanuelli C, Chao J, Regoli D, Chao L, Ni A, Madeddu P (1999). The bradykinin B1 receptor and the central regulation of blood pressure in spontaneously hypertensive rats. *Br J Pharmacol* **126**(8): 1769-1776.

Emerich DF, Dean RL, Osborn C, Bartus RT (2001). The development of the bradykinin agonist labradimil as a means to increase the permeability of the blood-brain barrier: from concept to clinical evaluation. *Clin Pharmacokinet* **40**(2): 105-123.

Emonds-Alt X, Vilain P, Goulaouic P, Proietto V, Van Broeck D, Advenier C (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK2) receptor. *Life Sci* **50**(15): PL101-106.

Emonds-Alt X, Bichon D, Ducoux JP, Heaulme M, Miloux B, Poncelet M, Proietto V, Van Broeck D, Vilain P, Neliat G (1995). SR 142801, the first potent non-peptide antagonist of the tachykinin NK3 receptor. *Life Sci* **56**(1):PL27-32.

Erdos EG, Yang HYT (1970). *Handbook of Experimental Pharmacology* Vol. 25, pp. 289-323, Springer-Verlag, New York.

Erdos EG (1990). Some old and some new ideas on kinin metabolism. *J Cardiovasc Pharmacol* **15**(6): S20-24.

Erdos EG, Skidgel RA (1989). Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J* **3**(2): 145-151.

Ernst M, Zametkin AJ, Phillips RL, Cohen RM (1998). Age-related changes in brain glucose metabolism in adults with attention-deficit/hyperactivity disorder and control subjects. *J Neuropsychiatry Clin Neurosci* **10**(2):168-177.

Erspamer V, Melchiorri P, Broccardo M, Erspamer GF, Falaschi P, Improta G (1981). The brain-gut-skin triangle: new peptides. *Peptides* **2**(2): 7-16.

Ewald DA, Pang IH, Sternweis PC, Miller RJ (1989). Differential G protein-mediated coupling of neurotransmitter receptors to Ca²⁺ channels in rat dorsal root ganglion neurons in vitro. *Neuron* **2**(2):1185-1193.

Fallon JH, Loughlin SE (1995). Substantia nigra. In: The Rat Nervous System, pp 215-237. Ed G. Paxinos. Academic Press: San Diego.

Farmer SG, Powell SJ, Wilkins DE, Graham A (1998). Cloning, sequencing and functional expression of a guinea pig lung bradykinin B2 receptor. *Eur J Pharmacol* **346**(2-3): 291-298.

Faussner A, Bathon JM, Proud D (1999). Comparison of the responses of B1 and B2 kinin receptors to agonist stimulation. *Immunopharmacology* **45**(1-3): 13-20.

Feldman PD (1995). Neurokinin1 receptor mediation of the vasodepressor effects of substance P in the nucleus of the tractus solitarius. *J Pharmacol Exp Ther.* **273**(2):617-23.

Fernandes L, Ceravolo GS, Fortes ZB, Tostes R, Santos RA, Santos JA (2006). Modulation of kinin B1 receptor expression by endogenous angiotensin II in hypertensive rats. *Regul Pept* **136**(1-3): 92-97.

Ferreira J, Campos MM, Pesquero JB, Araujo RC, Bader M, Calixto JB (2001). Evidence for the participation of kinins in Freund's adjuvant-induced inflammatory and nociceptive responses in kinin B1 and B2 receptor knockout mice. *Neuropharmacology* **41**(8): 1006-1012.

Fields HL, Hjelmstad GO, Margolis EB, Nicola SM (2007). Ventral tegmental area neurons in learned appetitive behaviour and positive reinforcement. *Annu Rev Neurosci* **30**: 289-316.

Finch L, Hausler G (1974). Vascular resistance and reactivity in hypertensive rats. *Blood Vessels* **11**:145-158.

Fior DR, Martins DT, Lindsey CJ (1993). Localization of central pressor action of bradykinin in medulla oblongata. *Am J Physiol* **265**(3 Pt 2): H1000-1006.

Fogaça SE, Melo RL, Pimenta DC, Hosoi K, Juliano L, Juliano MA (2004). Differences in substrate and inhibitor sequence specificity of human, mouse and rat tissue kallikreins. *Biochem J* **380**(Pt 3):775-781.

Frey EK (1926). Zusammenhänge zwischen Herzarbeit und Nierentätigkeit. *Arch Klim Chir* **142**: 663-669.

Frey U, Huang Y-Y, Kandel ER (1993). Effects of cAMP stimulate a late stage of LTP in hippocampal CA1 neurons. *Science* **260**:1661-1964.

Fuchs S, Frenzel K, Hubert C, Lyng R, Muller L, Michaud A (2005). Male fertility is dependent on dipeptidase activity of testis ACE. *Nat Med* **11**(11): 1140-1142; author reply 1142-1143.

Fujii T, Murai M, Morimoto H, Maeda Y, Yamaoka M, Hagiwara D (1992). Pharmacological profile of a high affinity dipeptide NK-1 receptor antagonist, FK888. *Br J Pharmacol* **107**(3): 785-789.

Fujiwara Y, Mantione CR, Yamamura HI (1988). Identification of B2 bradykinin binding sites in guinea-pig brain. *Eur J Pharmacol* **147**(3): 487-488.

Fujiwara Y, Mantione CR, Vavrek RJ, Stewart JM, Yamamura HI (1989). Characterization of [3H]bradykinin binding sites in guinea-pig central nervous system: possible existence of B2 subtypes. *Life Sci* **44**(22):1645-1653.

Furuto Kato S, Matsumoto A, Kitamura N, Nakanishi S (1985). Primary structures of the mRNAs encoding the rat precursors for bradykinin and T-kinin. Structural relationship of kininogens with major acute phase protein and alpha 1-cysteine proteinase inhibitor. *J Biol Chem* **260**:12054-12059.

Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC (1999). Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* **399**, 597-601.

Gabra BH, Benrezzak O, Pheng LH, Duta D, Daull P, Sirois P (2005a). Inhibition of type 1 diabetic hyperalgesia in streptozotocin-induced Wistar versus spontaneous gene-prone BB/Worchester rats: efficacy of a selective bradykinin B1 receptor antagonist. *J Neuropathol Exp Neurol* **64**(9): 782-789.

Gabra BH, Sirois P (2005b). Hyperalgesia in non-obese diabetic (NOD) mice: a role for the inducible bradykinin B1 receptor. *Eur J Pharmacol* **514**(1): 61-67.

Gabra BH, Sirois P (2003). Kinin B1 receptor antagonists inhibit diabetes-induced hyperalgesia in mice. *Neuropeptides* **37**(1): 36-44.

Gallacher DV, Hanley MR, Petersen OH, Roberts ML, Squire-Pollard LG, Yule DI (1990). Substance P and bombesin elevate cytosolic Ca^{2+} by different molecular mechanisms in a rat pancreatic acinar cell line. *J Physiol* **426**:193-207.

Ganju P, Davis A, Patel S, Nunez X, Fox A (2001). p38 stress-activated protein kinase inhibitor reverses bradykinin B(1) receptor-mediated component of inflammatory hyperalgesia. *Eur J Pharmacol* **421**(3): 191-199.

Garret C, Carruette A, Fardin V, Moussaoui S, Peyronel JF, Blanchard JC (1991). Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci U S A* **88**(22): 10208-10212.

Garrett RL, Brown JH (1972). Bradykinin interaction with 5-hydroxytryptamine, norepinephrine, and potassium chloride in rabbit aorta. *Proc Soc Exp Biol Med* **139**(4): 1344-1348.

Glatt CE, Snyder SH (1993). Cloning and expression of an adenylyl cyclase localized to the corpus striatum. *Nature* **361**(6412):536-538.

Gaudreau P, Barabe J, St-Pierre S, Regoli D (1981a). Pharmacological studies of kinins in venous smooth muscles. *Can J Physiol Pharmacol* **59**(4): 371-379.

Gaudreau P, Barabe J, St-Pierre S, Regoli D (1981b). Structure-activity study of kinins in vascular smooth muscles. *Can J Physiol Pharmacol* **59**(4): 380-389.

Gavioli EC, Canteras NS, De Lima TC (2002). The role of lateral septal NK-1 receptors in mediating anxiogenic effects induced by intracerebroventricular injection of substance P. *Behav Brain Res* **134**(1-2): 411-415.

Geisler S, Zahm DS (2005). Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol* **490**(3): 270-294.

Gerald WL, Chao J, Chao L (1986). Sex dimorphism and hormonal regulation of rat tissue kallikrein mRNA. *Biochim Biophys Acta* 867:16-23.

Gerard NP, Bao L, Xiao-Ping H, Gerard C (1993). Molecular aspects of the tachykinin receptors. *Regul Pept* 43(1-2):21-35.

Gervais J, Rouillard C (2000). Dorsal raphe stimulation differentially modulates dopaminergic neurons in the ventral tegmental area and SN. *Synapse* 35(4): 281-291.

Gobeil F, Filteau C, Pheng LH, Jukic D, Nguyen-Le XK, Regoli D (1996a). In vitro and in vivo characterization of bradykinin B2 receptors in the rabbit and the guinea pig. *Can J Physiol Pharmacol* 74(2): 137-144.

Gobeil F, Jr., Charland S, Filteau C, Perron SI, Neugebauer W, Regoli D (1999). Kinin B1 receptor antagonists containing alpha-methyl-L-phenylalanine: in vitro and in vivo antagonistic activities. *Hypertension* 33(3): 823-829.

Gobeil F, Jr., Montagne M, Inamura N, Regoli D (1999b). Characterization of non-peptide bradykinin B2 receptor agonist (FR 190997) and antagonist (FR 173657). *Immunopharmacology* 43(2-3): 179-185.

Gobeil F, Neugebauer W, Filteau C, Jukic D, Allogho SN, Pheng LH (1996b). Structure-activity studies of B1 receptor-related peptides. Antagonists. *Hypertension* 28(5): 833-839.

Goldberg MR, Chapnick BM, Joiner PD, Hyman AL, Kadowitz PJ (1976). Influence of inhibitors of prostaglandin synthesis on venoconstrictor responses to bradykinin. *J Pharmacol Exp Ther* 198(2): 357-365.

Goodman LS, Gilman AG (1996). *The Pharmacological Basis of Therapeutics*. Ninth. McGraw-Hill.

Gougat J, Ferrari B, Sarran L, Planchenault C, Poncelet M, Maruani J (2004). SSR240612 [(2R)-2-[(3R)-3-(1,3-benzodioxol-5-yl)-3-[(6-methoxy-2-naphthyl)sulfonyl]amino]propanoyl]amino]-3-(4-[[2R,6S)-2,6-dimethylpiperidinyl]methyl]phenyl)-N-isopropyl-N-methylpropanamide hydrochloride], a new nonpeptide antagonist of the bradykinin B1 receptor: biochemical and pharmacological characterization. *J Pharmacol Exp Ther* 309(2): 661-669.

Grady EF, Garland AM, Gamp PD, Lovett M, Payan DG, Bunnett NW (1995). Delineation of the endocytic pathway of substance P and its seven-transmembrane domain NK-1 receptor. *Mol Biol Cell* **6**(5): 509-524.

Grandordy BM, Frossard N, Rhoden KJ, Barnes PJ (1988). Tachykinin-induced phosphoinositide breakdown in airway smooth muscle and epithelium: relationship to contraction. *Mol Pharmacol* **33**(5):515-519.

Grassi G (1998). Role of the sympathetic nervous system in human hypertension. *J Hypertens* **16**(12 Pt 2): 1979-1987.

Griebel G, Perrault G, Soubrie P (2001). Effects of SR48968, a selective non-peptide NK2 receptor antagonist on emotional processes in rodents. *Psychopharmacology (Berl)* **158**:241-251.

Griesbacher T, Amann R, Sametz W, Diethart S, Juan H (1998). The nonpeptide B2 receptor antagonist FR173657: inhibition of effects of bradykinin related to its role in nociception. *Br J Pharmacol* **124**(6): 1328-1334.

Griesbacher T, Legat FJ (2000). Effects of the non-peptide B2 receptor antagonist FR173657 in models of visceral and cutaneous inflammation. *Inflamm Res* **49**(10): 535-540.

Griesbacher T, Sametz W, Legat FJ, Diethart S, Hammer S, Juan H (1997). Effects of the non-peptide B2 antagonist FR173657 on kinin-induced smooth muscle contraction and relaxation, vasoconstriction and prostaglandin release. *Br J Pharmacol* **121**(3): 469-476.

Guard S, Watson SP (1991). Tachykinin receptor subtypes: classification and membrane signalling mechanisms. *Neurochem Int* **18**: 149-165.

Gysling K, Wang RY (1983). Morphine-induced activation of A10 dopamine neurons in the rat. *Brain Res* **277**(1): 119-127.

Habermann E (1963). Über pH-bedingte Modifikationen des Kininliefernden γ -Globulins (Kininogen) aus Rinderserum und das Molekulargewicht von Kininogen I. *Biochem Z* **337**: 440-448.

Haddad EB, Fox AJ, Rousell J, Burgess G, McIntyre P, Barnes PJ (2000). Post-transcriptional regulation of bradykinin B1 and B2 receptor gene

expression in human lung fibroblasts by tumor necrosis factor- α : modulation by dexamethasone. *Mol Pharmacol* **57**(6): 1123-1131.

Hagan RM, Ireland SJ, Jordan CC, Beresford IJ, Deal MJ, Ward P (1991). Receptor-selective, peptidase-resistant agonists at neurokinin NK-1 and NK-2 receptors: new tools for investigating neurokinin function. *Neuropeptides* **19**(2): 127-135.

Hall H, Ogren SO, Kohler C, Magnusson O (1989). Animal pharmacology of raclopride, a selective dopamine D2 antagonist. *Psychopharmacol Ser* **7**: 123-130.

Halliday GM, Tork I (1986). Comparative anatomy of the ventromedial mesencephalic tegmentum in the rat, cat, monkey and human. *J Comp Neurol* **252**(4): 423-445.

Harrison S, Geppetti P (2001). Substance P. *Int J Biochem Cell Biol* **33**(6): 555-576.

Hashimoto K, Ishiwata K (2006). Sigma receptor ligands: possible application as therapeutic drugs and as radiopharmaceuticals. *Curr Pharm Des* **12**(30): 3857-3876.

Hassessian H, Couture R, de Champlain J (1990). Sympathoadrenal mechanisms underlying cardiovascular responses to intrathecal substance P in conscious rats. *J Cardiovasc Pharmacol* **15**(5): 736-744.

Häusler G (1982). Central alpha-adrenoceptors involved in cardiovascular regulation. *J Cardiovasc Pharmacol* **4**: (Suppl 1) S72-76.

Hay DW, Giardina GA, Griswold DE, Underwood DC, Kotzer CJ, Bush B (2002). Nonpeptide tachykinin receptor antagonists. III. SB 235375, a low central nervous system-penetrant, potent and selective neurokinin-3 receptor antagonist, inhibits citric acid-induced cough and airways hyper-reactivity in guinea pigs. *J Pharmacol Exp Ther* **300**(1): 314-323.

Haxhiu MA, Dreshaj I, Schäfer SG, Ernsberger P (1994). Selective antihypertensive action of moxonidine is mediated mainly by I1-imidazoline receptors in the rostral ventrolateral medulla. *J Cardiovasc Pharmacol* **24**: (Suppl 1) S1-8.

Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience* **41**(1): 89-125.

Heise A, Kroneberg G (1972). α -Sympathetic receptor stimulation in the brain and the hypotensive action of α -methyldopa. *Eur J Pharmac* **17**: 315-317.

Helke CJ, O'Donohue TL, Jacobowitz DM (1980). Substance P as a baro- and chemoreceptor afferent neurotransmitter: immunocytochemical and neurochemical evidence in the rat. *Peptides*. 1980 Spring;1(1):1-9.

Helke CJ, Seagard JL (2004). Substance P in the baroreceptor reflex: 25 years. *Peptides* **25**(3): 413-423.

Henning M, van Zwieten PA (1968). Central hypotensive effect of α -methyldopa. *J Pharm Pharmacol* **20**(6):409-17.

Henry J, Couture R, Cuello AC, Pelletier G, Quirion R, Regoli D (1987). Substance P and Neurokinins. Proceedings of a satellite symposium of the XXX International Congress of Physiological Sciences held in Montréal, July 21-23, 1986, Springer-Verlag, New York, pp. 450.

Hershey AD, Krause JE (1990). Molecular characterization of a functional cDNA encoding the rat substance P receptor. *Science* **247**(4945): 958-962.

Herve D, Pickel VM, Joh TH, Beaudet A (1987). Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. *Brain Res* **435**(1-2): 71-83.

Hess JF, Derrick AW, MacNeil T, Borkowski JA (1996). The agonist selectivity of a mouse B1 bradykinin receptor differs from human and rabbit B1 receptors. *Immunopharmacology* **33**(1-3):1-8.

Hess JF, Borkowski HA, Young GS, Strader CD, Ramsom RW (1992). Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem Biophys Res Commun* **184**: 260-268.

Hess JF, Borkowski JA, Macneil T, Stonesifer GY, Fraher J, Strader CD (1994). Differential pharmacology of cloned human and mouse B2 bradykinin receptors. *Mol Pharmacol* **45**(1): 1-8.

Hess JF, Hey PJ, Chen TB, O'Brien J, Omalley SS, Pettibone DJ (2001). Molecular cloning and pharmacological characterization of the canine B1 and B2 bradykinin receptors. *Biol Chem* **382**(1): 123-129.

Hildebrand KR, Ujhelyi MR, Zhou X, Sigg DC, Page LM (2005). Delivery of a sympatholytic cardiovascular agent to the central nervous system. www.faq.s.org/patents/app/20080228133.

Higashida H, Streaty RA, Klee W, Nirenberg M (1986). Bradykinin-activated transmembrane signals are coupled via No or Ni to production of inositol 1,4,5-trisphosphate, a second messenger in NG108-15 neuroblastoma-glioma hybrid cells. *Proc Natl Acad Sci USA* **83**(4):942-946.

Hock FJ, Wirth K, Albus U, Linz W, Gerhards HJ, Wiemer G (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol* **102**(3): 769-773.

Hoffman BB (2006). "Therapy of Hypertension" in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition, (Brunton LL, Lazo JS and Parker KL, eds) McGraw-Hill Medical Publishing Division, NewYork.

Hökfelt T, Elde R, Johansson O, Luft R, Nilsson G, Arimura A (1976). Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. *Neuroscience* **1**(2):131-136.

Hokfelt T, Ljungdahl A, Terenius L, Elde R, Nilsson G (1977). Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: enkephalin and substance P. *Proc Natl Acad Sci U S A* **74**(7): 3081-3085.

Hsieh HL, Wu CY, Hwang TL, Yen MH, Parker P, Yang CM (2006). BK-induced cytosolic phospholipase A2 expression via sequential PKC-delta,

p42/p44 MAPK, and NF-kappaB activation in rat brain astrocytes. *J Cell Physiol* **206**(1):246-54.

Hunter JC, Goedert M, Pinnock RD (1985). Mammalian tachykinin-induced hydrolysis of inositol phospholipids in rat brain slices. *Biochem Biophys Res Commun*.127(2):616-22.

Huston JP, Hasenohrl RU (1995). The role of neuropeptides in learning: focus on the neurokinin substance P. *Behav Brain Res* **66**(1-2): 117-127.

Ignjatovic T, Stanisavljevic S, Brovkovich V, Skidgel RA, Erdos EG (2004). Kinin B1 receptors stimulate nitric oxide production in endothelial cells: signaling pathways activated by angiotensin I-converting enzyme inhibitors and peptide ligands. *Mol Pharmacol* **66**(5):1310-1316.

Inamura N, Asano M, Kayakiri H, Hatori C, Oku T, Nakahara K (1997). Characterization of FR173657, a novel nonpeptide B2 antagonist: in vitro and in vivo studies. *Can J Physiol Pharmacol* **75**(6): 622-628.

Innis RB, Manning DC, Stewart JM, Snyder SH (1981). [3H] bradykinin receptor binding in mammalian tissue membranes. *Proc Nat Acad Sci USA* **78**: 2630-2634.

Iorio LC, Barnett A, Leitz FH, Houser VP, Korduba CA (1983). SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. *J Pharmacol Exp Ther* **226**(2):462-468.

Israel A, Saavedra JM (1987). High angiotensin converting enzyme (kininase II) activity in the cerebrospinal fluid of spontaneously hypertensive adult rats. *J Hypertens* **5**:355-357.

Itoi K, Jost N, Badoer E, Tschöpe C, Culman J, Unger T (1991). Localization of the substance P-induced cardiovascular responses in the rat hypothalamus. *Brain Res* **558**(1):123-126.

Itoi K, Tschöpe C, Jost N, Culman J, Lebrun C, Stauss B, Unger T (1992). Identification of the central tachykinin receptor subclass involved in substance P-induced cardiovascular and behavioural responses in conscious rats. *Eur J Pharmacol* **219**(3):435-444.

Itoi K, Jost N, Tschöpe C, Culman J, Badoer E, Unger T. (1994). Inhibition by morphine of the cardiovascular and behavioural responses evoked by centrally administered substance P in conscious rats. *Neuropharmacology* **33**(2):181-187.

Iwadata H, Kawamata K, Kudo M, Kizuki K (2000). Biochemical and immunohistochemical demonstration of tissue kallikrein in the neuronal nuclei of the developing rat brains. *Brain Res* **863**(1-2): 87-93.

Iyengar S, Hipskind PA, Gehlert DR, Schober D, Lobb KL, Nixon JA (1997). LY303870, a centrally active neurokinin-1 antagonist with a long duration of action. *J Pharmacol Exp Ther* **280**(2): 774-785.

Jackson DM, Westlind-Danielsson A (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol Ther* **64**(2): 291-370.

Johanson CE, Duncan JA, Stopa EG, Baird A (2005). *Pharmaceutical Research* **22**(7)1011-1037.

Johnson SW, North RA (1992b). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol* **450**: 455-468.

Julius S, Schork N, Schork A (1988). Sympathetic hyperactivity in early stages of hypertension: the Ann Arbor data set. *J Cardiovasc Pharmacol* **12 Suppl 3**: S121-129.

Jones S, Brown DA, Milligan G, Willer E, Buckley NJ, Caulfield MP (1995). Bradykinin excites rat sympathetic neurons by inhibition of M current through a mechanism involving B2 receptors and G alpha q/11. *Neuron* **14**(2):399-405.

Jones C, Phillips E, Davis C, Arbuckle J, Yaqoob M, Burgess GM, Docherty RJ, Webb M, Bevan SJ, McIntyre P (1999). Molecular characterisation of cloned bradykinin B1 receptors from rat and human. *Eur J Pharmacol* **374**(3):423-433.

Jung AB, Bennett JP, Jr (1996). Development of striatal dopaminergic function. I. Pre- and postnatal development of mRNAs and binding sites for striatal D1 (D1a) and D2 (D2a) receptors. *Brain Res Dev Brain Res* **94**(2): 109-120.

Kaczyńska K, Szereda-Przestaszewska M (2006). Clonidine-evoked respiratory effects in anaesthetized rats. *Exp Physiol* **91**(1):269-275.

Kage R, McGregor GP, Thim L, Conlon JM (1988). Neuropeptide-gamma: a peptide isolated from rabbit intestine that is derived from gamma-preprotachykinin. *J Neurochem* **50**(5): 1412-1417.

Kalivas PW, Churchill L, Klitenick MA (1993). GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. *Neuroscience* **57**(4): 1047-1060.

Kalivas PW, Deutch AY, Maggio JE, Mantyh PW, Roth RH (1985). Substance K and substance P in the ventral tegmental area. *Neurosci Lett* **24**; 57(3): 241-246.

Kammerer S, Braun A, Arnold N, Roscher AA (1995). The human bradykinin B2 receptor gene: full length cDNA, genomic organization and identification of the regulatory region. *Biochem Biophys Res Commun* **211**(1): 226-233.

Kanazawa I, Jessell T (1976). Post mortem changes and regional distribution of substance P in the rat and mouse nervous system. *Brain Res* **117**(2): 362-367.

Kangawa K, Minamino N, Fukuda A, Matsuo H (1983). Neuromedin K: a novel mammalian tachykinin identified in porcine spinal cord. *Biochem Biophys Res Commun* **114**(2): 533-540.

Kaplan AP, Joseph K, Shibayama Y (1997). Binding of activation of kinin-forming proteins on vascular endothelial cells. *Immunopharmacology* **36**(2-3): 201-207.

Kariya K, Kawauchi R, Okamoto H (1981). Regional distribution of kininase in rat brain. *J Neurochem* **36**(6): 2086-2088.

Kariya K, Yamauchi A, Sasaki T (1985). Regional distribution and characterization of kinin in the CNS of the rat. *J Neurochem* **44**(6): 1892-1897.

Kaufman JM, Vermeulen A (1989). Lack of effect of the alpha-adrenergic agonist clonidine on pulsatile luteinizing hormone secretion in a double blind study in men. *J Clin Endocrinol Metab* **68**(1): 219-222.

Kebabian JW, Calne DB (1979). Multiple receptors for dopamine. *Nature* **277**(5692):93-96.

Kertes E, Laszlo K, Berta B, Lenard L (2009). Effects of substance P microinjections into the globus pallidus and central nucleus of amygdala on passive avoidance learning in rats. *Behav Brain Res* **198**(2): 397-403.

Khasar SG, Miao FJ, Levine JD (1995). Inflammation modulates the contribution of receptor-subtypes to bradykinin-induced hyperalgesia in the rat. *Neuroscience* **69**(2): 685-690.

Khawaja AM, Rogers DF (1996). Tachykinins: receptor to effector. *Int J Biochem Cell Biol* **28**(7):721-738.

Kimura T, Share L, Wang BC, Crofton J (1981). The role of central adrenoreceptors in the control of vasopressin release and blood pressure. *Endocrinology* **108**(5):1829-1836.

Kimura S, Goto K, Ogawa T, Sugita Y, Kanazawa I (1984). Pharmacological characterization of novel mammalian tachykinins, neurokinin alpha and neurokinin beta. *Neurosci Res* **2**(1-2): 97-104.

Kintsurashvili E, Duka I, Gavras I, Johns C, Farmakiotis D, Gavras H (2001). Effects of ANG II on bradykinin receptor gene expression in cardiomyocytes and vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* **281**(4): H1778-1783.

Kirouac GJ, Ganguly PK (1993). Up-regulation of dopamine receptors in the brain of the spontaneously hypertensive rat: an autoradiographic analysis. *Neuroscience* **52**(1):135-141.

Kirouac GJ, Ciriello J (1997). Cardiovascular depressor responses to stimulation of substantia nigra and ventral tegmental area. *Am J Physiol* **273**(6 Pt 2):H2549-2557.

Kizuki K, Suzuki T, Kudo M, Noguchi T (1994). Immunohistochemical demonstration of tissue kallikrein in the neurons of rat brain. *Brain Res* **634**(2): 305-309.

Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP (2001). Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J Neurosci* **21**(5): 1452-1463.

Kobinger G (1978). Central alpha-adrenergic systems as targets for hypotensive drugs. *Rev Physiol Biochem Pharmacol* **81**:39-100.

Koss MC (1983). Analysis of CNS sympatho-inhibition produced by guanabenz. *Eur J Pharmacol* **90**(1):19-27.

Korner PI (1989). Baroreceptor resetting and other determinants of baroreflex properties in hypertension. *Clin Exp Pharmacol Physiol Suppl* **15**: 45-64.

Korner PI, Bobik A, Angus JA, Adams MA, Friberg P (1989). Resistance control in hypertension. *J Hypertens Suppl* **7**(4): S125-134; discussion S135.

Kosman ME (1975). Evaluation of clonidine hydrochloride (Catapres). A new antihypertensive agent. *JAMA*. **233**(2):174-176.

Kozlowski MR, Rosser MP, Hall E (1988). Identification of 3H-bradykinin binding sites in PC-12 cells and brain. *Neuropeptides* **12**(4):207-211.

Kramer MS, Cutler N, Feighner J, Shrivastava R, Carman J, Sramek JJ (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* **281**(5383): 1640-1645.

Kraut H, Frey EK, Werle E (1930). Über die Inaktivierung des Kallikreins. *Hoppe-Seyler's Z Physiol Chem* **192**: 1 - 21.

Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987). Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. *Proc Natl Acad Sci U S A* **84**(3): 881-885.

Krstew E, Jarrott B, Lawrence AJ (1998). Bradykinin B2 receptors in nodose ganglia of rat and human. *Eur J Pharmacol* **348**(2-3): 175-180.

Kubo T, Kihara M (1987). Blood pressure modulation by substance P in the rat nucleus tractus solitarius. *Brain Res* **413**(2):379-383.

Kubos KL, Moran TH, Robinson RG (1987). Differential and asymmetrical behavioural effects of electrolytic or 6-hydroxydopamine lesions in the nucleus accumbens. *Brain Res* **401**(1): 147-151.

Kubo T, Misu Y (1981) Pharmacological characterization of the alpha-adrenoreceptors responsible for a decrease of blood pressure in the nucleus tractus solitarii of the rat. *Naunyn-Schmiedeberg's Arch Pharmacol* **317**:120-125.

Kuhr F, Lowry J, Zhang Y, Brovkovich V, Skidgel RA (2010). Differential regulation of inducible and endothelial nitric oxide synthase by kinin B1 and B2 receptors. *Neuropeptides* **44**(2):145-54.

Laflamme K, de Champlain J (1998a). [Interaction between the sympathetic and renin-angiotensin system]. *Therapie* **53**(3): 245-252.

Laflamme K, Wu L, Foucart S, de Champlain J (1998b). Impaired basal sympathetic tone and alpha1-adrenergic responsiveness in association with the hypotensive effect of melatonin in spontaneously hypertensive rats. *Am J Hypertens* **11**(2): 219-229.

Lagneux C, Innocenti-Francillard P, Godin-Ribuot D, Bader M, Ribuo C (1998). Heat stress-induced B1 receptor synthesis in the rat: an ex vivo study. *Br J Pharmacol* **125**(4): 812-816.

Lagneux C, Ribuo C (1997). In vivo evidence for B1-receptor synthesis induction by heat stress in the rat. *Br J Pharmacol* **121**(6): 1045-1046.

Lahti RA, Roberts RC, Tamminga CA (1995). D2-family receptor distribution in human postmortem tissue: an autoradiographic study. *Neuroreport* **6**(18): 2505-2512.

Lalli E, Sassone-Corsi P, Ceredig R (1996). Block of T lymphocyte differentiation by activation of the cAMP-dependent signal transduction pathway. *EMBO J* **15**(3): 528-537.

Laniyonu A, Sliwinski-Lis E, Fleming N (1988). Different tachykinin receptor subtypes are coupled to the phosphoinositide or cyclic AMP signal transduction pathways in rat submandibular cells. *FEBS Lett* **240**(1-2):186-190.

Laneuville O, Dorais J, Couture R (1988). Characterization of the effects produced by neurokinins and three agonists selective for neurokinin receptor subtypes in a spinal nociceptive reflex of the rat. *Life Sci* **42**(13): 1295-1305.

Langlois X, Wintmolders C, te Riele P, Leysen JE, Jurzak M (2001). Detailed distribution of Neurokinin 3 receptors in the rat, guinea pig and gerbil brain: a comparative autoradiographic study. *Neuropharmacology* **40**(2): 242-253.

Laplanche MA, Wu R, El Midaoui A, de Champlain J (2003). NAD(P)H oxidase activation by angiotensin II is dependent on p42/44 ERK-MAPK pathway activation in rat's vascular smooth muscle cells. *J Hypertens* **21**: 927-936.

Larsen PJ (1992). Distribution of substance P-immunoreactive elements in the preoptic area and the hypothalamus of the rat. *J Comp Neurol* **316**(3): 287-313.

Laruelle M, Kegeles LS, Abi-Dargham A (2003). Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. *Ann N Y Acad Sci* **1003**: 138-158.

Lawrence ID, Warner JA, Cohan VL, Lichtenstein LM, Kagey-Sobotka A, Vavrek RJ (1989). Induction of histamine release from human skin mast cells by bradykinin analogs. *Biochem Pharmacol* **38**(2): 227-233.

Lebel M, Robinson P, Cyr M (2007). Canadian Association of Neurosciences Review: the role of dopamine receptor function in neurodegenerative diseases. *Can J Neurol Sci* **34**(1): 18-29.

Leeb-Lundberg LM, Kang DS, Lamb ME, Fathy DB (2001). The human B1 bradykinin receptor exhibits high ligand-independent, constitutive activity. Roles of residues in the fourth intracellular and third transmembrane domains. *J Biol Chem* **276**(12):8785-92.

Leeb-Lundberg LM, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL (2005). International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol Rev* **57**(1): 27-77.

Lee CM, Tung WL and Young JD (1992) Tachykinin-stimulated inositol phospholipid hydrolysis and taurine release from human astrocytoma cells. *J Neurochem* **59**: 406–414.

Lee YJ, Zachrisson O, Tonge DA, McNaughton PA (2002). Upregulation of bradykinin B2 receptor expression by neurotrophic factors and nerve injury in mouse sensory neurons. *Mol Cell Neurosci* **19**(2):186-200.

Le Fur G, Guilloux F, Kabouche M, Mitrani N, Ferris O, Uzan A (1981). Central dopaminergic neurons during development of genetic and DOCA-salt hypertension in the rat. *Brain Res* **227**(2):153-163.

Legault M, Congar P, Michel FJ, Trudeau LE (2002). Presynaptic action of neurotensin on cultured ventral tegmental area dopaminergic neurones. *Neuroscience*. 111(1):177-87.

Lessard A, Campos MM, Neugebauer W, Couture R (2003). Implication of nigral tachykinin NK-3Rs in the maintenance of hypertension in spontaneously hypertensive rats: a pharmacologic and autoradiographic study. *Br J Pharmacol* **138**(4): 554-563.

Lessard A, Couture R (2001). Modulation of cardiac activity by tachykinins in the rat SN. *Br J Pharmacol* **134**(8): 1749-1759.

Lessard A, Grady EF, Bunnett NW, Pickel VM (2007). Predominant surface distribution of neurokinin-3 receptors in non-dopaminergic dendrites in the rat SN and ventral tegmental area. *Neuroscience* **144**(4): 1393-1408.

Lessard A, Laurin M, Yamaguchi N, Couture R (2004). Central anti-hypertensive effect of tachykinin NK-3R antagonists in rat. *Eur J Pharmacol* **486**(1): 75-83.

Lessard A, Savard M, Gobeil F, Jr., Pierce JP, Pickel VM (2009). The neurokinin-3 (NK3) and the neurokinin-1 (NK-1) receptors are differentially targeted to mesocortical and mesolimbic projection neurons and to neuronal nuclei in the rat ventral tegmental area. *Synapse* **63**(6): 484-501.

Leung DY (1998). Molecular basis of allergic diseases. *Mol Genet Metab* **63**(3): 157-167.

Levesque L, Harvey N, Rioux F, Drapeau G, Marceau F (1995). Development of a binding assay for the B1 receptors for kinins. *Immunopharmacology* **29**(2):141-147.

Lewis RE, Phillips MI (1984). Localization of the central pressor action of bradykinin to the cerebral third ventricle. *Am J Physiol* **247**(1 Pt 2): R63-68.

Lewis RE, Childers SR, Phillips MI. [125I]Tyr-bradykinin binding in primary rat brain cultures. *Brain Res* **346**(2):263-272.

Liebmann C, Böhmer FD (2000). Signal transduction pathways of G protein-coupled receptors and their cross-talk with receptor tyrosine kinases: lessons from bradykinin signaling. *Curr Med Chem* **7**(9):911-943.

Liebmann C (2001). Bradykinin signalling to MAP kinase: cell-specific connections versus principle mitogenic pathways. *Biol Chem* **382**(1):49-55.

Lightman SL, Young WS (1988). III Corticotropin-releasing factor, vasopressin and pro-opiomelanocortin mRNA responses to stress and opiates in the rat. *The Journal of Physiology* **403**:511-523.

Linden DR, Jia YP, Seybold VS (1999). Spinal neurokinin (3) receptors facilitate the nociceptive flexor reflex via a pathway involving nitric oxide. *Pain* **80**(1-2): 301-308.

Lindsey CJ, Buck HS, Fior-Chadi DR, Lapa RC (1997). Pressor effect mediated by bradykinin in the paratrigeminal nucleus of the rat. *J Physiol* **502** (Pt 1): 119-129.

Lindsey CJ, Fujita K, Martins TO (1988). The central pressor effect of bradykinin in normotensive and hypertensive rats. *Hypertension* **11**(2 Pt 2): I126-129.

Lindsey CJ, Nakaie CR, Martins DT (1989). Central nervous system kinin receptors and the hypertensive response mediated by bradykinin. *Br J Pharmacol* **97**(3): 763-768.

Lindstrom E, von Mentzer B, Pahlman I, Ahlstedt I, Uvebrant A, Kristensson E (2007). Neurokinin 1 receptor antagonists: correlation between in vitro receptor interaction and in vivo efficacy. *J Pharmacol Exp Ther* **322**(3): 1286-1293.

Linthorst AC, van Giersbergen PL, Gras M, Versteeg DH, de Jong W (1994). The nigrostriatal dopamine system: role in the development of hypertension in spontaneously hypertensive rats. *Brain Res* **639**(2):261-268.

Linz W, Wiemer G, Gohlke P, Unger T, Scholkens BA (1995). Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev* **47**(1): 25-49.

Liss B, Roeper J (2008). Individual dopamine midbrain neurons: functional diversity and flexibility in health and disease. *Brain Res Rev* **58**(2): 314-321.

Ljungdahl A, Hokfelt T, Nilsson G (1978). Distribution of substance P-like immunoreactivity in the central nervous system of the rat--I. Cell bodies and nerve terminals. *Neuroscience* **3**(10): 861-943.

Lopes P, Couture R (1992). Cardiovascular responses elicited by intrathecal kinins in the conscious rat. *Eur J Pharmacol* **210**(2): 137-147.

Lopes P, Couture R (1997). Localization of bradykinin-like immunoreactivity in the rat spinal cord: effects of capsaicin, melittin, dorsal rhizotomy and peripheral axotomy. *Neuroscience* **78**(2): 481-497.

Lopes P, Kar S, Chrétien L, Regoli D, Quirion R, Couture R (1995). Quantitative autoradiographic localization of [125I-Tyr8]bradykinin receptor binding sites in the rat spinal cord: effects of neonatal capsaicin, noradrenergic deafferentation, dorsal rhizotomy and peripheral axotomy. *Neuroscience* **68**(3):867-881.

Lopes P, Regoli D, Couture R (1993). Cardiovascular effects of intrathecally administered bradykinin in the rat: characterization of receptors with antagonists. *Br J Pharmacol* **110**(4): 1369-1374.

Lopes P, Kar S, Tousignant C, Regoli D, Quirion R, Couture R (1993). Autoradiographic localization of [125I-Tyr8]-bradykinin receptor binding sites in the guinea pig spinal cord. *Synapse* **15**(1):48-57.

Lu XY, Ghasemzadeh MB, Kalivas PW (1998). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* **82**: 767-780.

Lucas LR, Hurley DL, Krause JE, Harlan RE (1992). Localization of the tachykinin neurokinin B precursor peptide in rat brain by immunocytochemistry and in situ hybridization. *Neuroscience* **51**(2): 317-345.

Lungu C, Dias JP, Franca CE, Ongali B, Regoli D, Moldovan F (2007). Involvement of kinin B1 receptor and oxidative stress in sensory abnormalities and arterial hypertension in an experimental rat model of insulin resistance. *Neuropeptides* **41**(6): 375-387.

Ma JX, Wang DZ, Chao L, Chao J (1994a). Cloning, sequence analysis and expression of the gene encoding the mouse bradykinin B2 receptor. *Gene* **149**(2): 283-288.

Ma JX, Wang DZ, Ward DC, Chen L, Dessai T, Chao J (1994b). Structure and chromosomal localization of the gene (BDKRB2) encoding human bradykinin B2 receptor. *Genomics* **23**(2): 362-369.

Ma QP, Tian L (2001). A-fibres sprouting from laminae I into laminae II of spinal dorsal horn after peripheral nerve injury in rats. *Brain Res* **904**(1): 137-140.

MacNeil T, Bierilo KK, Menke JG, Hess JF (1995). Cloning and pharmacological characterization of a rabbit bradykinin B1 receptor. *Biochim Biophys Acta* **1264**(2):223-228.

Madeddu P, Glorioso N, Varoni MV, Demontis MP, Fattaccio MC, Anania V (1994). Cardiovascular effects of brain kinin receptor blockade in spontaneously hypertensive rats. *Hypertension* **23**(1):189-92.

Maggi CA, Patacchini R, Eglezos A, Quartara L, Giuliani S, Giachetti A (1992). Tachykinin receptors in the guinea-pig renal pelvis: activation by exogenous and endogenous tachykinins. *Br J Pharmacol* **107**(1): 27-33.

Maggi CA, Astolfi M, Giuliani S, Goso C, Manzini S, Meini S, Patacchini R, Pavone V, Pedone C, Quartara L (1994). MEN 10,627, a novel polycyclic peptide antagonist of tachykinin NK2 receptors. *J Pharmacol Exp Ther* **1**(3):1489-1500.

Maggi CA (1995). The mammalian tachykinin receptors. *Gen Pharmacol* **26**(5): 911-944.

Maggi CA (2000). The troubled story of tachykinins and neurokinins. *Trends Pharmacol Sci* **21**(5): 173-175.

Magoul R, Onteniente B, Oblin A, Calas A (1986). Inter- and intracellular relationship of substance P-containing neurons with serotonin and GABA in the dorsal raphe nucleus: combination of autoradiographic and immunocytochemical techniques. *J Histochem Cytochem* **34**(6): 735-742.

Mah GT, Tejani AM, Musini VM (2009). Methyldopa for primary hypertension. *Cochrane Database of Systematic Reviews* Issue 4.

Mai JK, Stephens PH, Hopf A, Cuello AC (1986). Substance P in the human brain. *Neuroscience* **17**(3): 709-739.

Majima M, Kawashima N, Hiroshi I, Katori M (1997). Effects of an orally active non-peptide bradykinin B2 receptor antagonist, FR173657, on plasma exudation in rat carrageenin-induced pleurisy. *Br J Pharmacol* **121**(4):723-730.

Mantyh PW, Pinnock RD, Downes CP, Goedert M, Hunt SP (1984). *Nature* **309**(5971):795-7.

Marceau F, Barabe J, St-Pierre S, Regoli D (1980). Kinin receptors in experimental inflammation. *Can J Physiol Pharmacol* **58**(5): 536-542.

Marceau F (1995). Kinin B1 receptors: a review. *Immunopharmacology* **30**(1): 1-26.

Marceau F, Adam A, Houle S, Bouthillier J, Bachvarova M, Bachvarov DR (2001). Ligand-mediated regulation of kinin receptors in the rabbit. *Biol Chem* **382**(1): 131-133.

Marceau F, Bachvarov DR (1998a). Kinin receptors. *Clin Rev Allergy Immunol* **16**(4): 385-401.

Marceau F, Hess JF, Bachvarov DR (1998b). The B1 receptors for kinins. *Pharmacol Rev* **50**(3): 357-386.

Marceau F, Lussier A, St-Pierre S (1984). Selective induction of cardiovascular responses to des-Arg⁹-bradykinin by bacterial endotoxin. *Pharmacology* **29**(2): 70-74.

Marcic B, Deddish PA, Jackman HL, Erdos EG (1999). Enhancement of bradykinin and resensitization of its B2 receptor. *Hypertension* **33**(3): 835-843.

Marcic BM, Erdos EG (2000). Protein kinase C and phosphatase inhibitors block the ability of angiotensin I-converting enzyme inhibitors to resensitize the receptor to bradykinin without altering the primary effects of bradykinin. *J Pharmacol Exp Ther* **294**:605-612.

Marco N, Thirion A, Mons G, Bougault I, Le Fur G, Soubrie P (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK-3R stimulation: an in vivo microdialysis approach in guinea pig. *Neuropeptides* **32**(5): 481-488.

Marks N, Stern F, Chi LM, Berg MJ (1988). Diversity of rat brain cysteine proteinase inhibitors: isolation of low-molecular-weight cystatins and a higher-molecular weight T-kininogen-like glycoprotein. *Arch Biochem Biophys* **267**(2):448-458.

Martins DT, Fior DR, Nakaie CR, Lindsey CJ (1991). Kinin receptors of the central nervous system of spontaneously hypertensive rats related to the pressor response to bradykinin. *Br J Pharmacol* **103**(4): 1851-1856.

Mason GS, Elliott PJ (1992). Behavioural consequences following infusion of selective neurokinin agonists into the median raphe nucleus of the rat. *Neuropharmacology* **31**(8): 757-760.

Mathis SA, Criscimagna NL, Leeb-Lundberg LM (1996). B1 and B2 kinin receptors mediate distinct patterns of intracellular Ca²⁺ signaling in single cultured vascular smooth muscle cells. *Mol Pharmacol* **50**(1):128-139.

Matsuoka I, Suzuki Y., Defer N., Nakanishi H., Hanoune J (1997). Differential expression of type I, II, and V adenylyl cyclase gene in the postnatal developing rat brain. *J Neurochem* **68**:498-506.

McCubbin JW, Kaneko Y, Page IH (1960). Ability of serotonin and norepinephrine to mimic the central effects of reserpine on vasomotor activity. *Circulation Research* **8**: 849-851.

McEachern AE, Shelton ER, Bharkta S, Obernolte R, Bach C, Zuppan P, Fujisaki J, Aldrich RW, Jarnagin K (1991). Expression cloning of a rat B2 bradykinin receptor. *Proc Natl Acad Sci USA* **88**:7724-7728.

McFarland K, Kalivas PW (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behaviour. *J Neurosci* **21**(21): 8655-8663.

McGiff JC, Itskovitz HD, Terragno A, Wong PY (1976). Modulation and mediation of the action of the renal kallikrein-kinin system by prostaglandins. *Fed Proc* **35**(2): 175-180.

Meador-Woodruff JH, Mansour A, Civelli O, Watson SJ (1991). Distribution of D2 dopamine receptor mRNA in the primate brain. *Prog Neuropsychopharmacol Biol Psychiatry* **15**(6):885-893.

Meador-Woodruff JH, Mansour A, Grandy DK, Damask SP, Civelli O, Watson SJ Jr (1992). Distribution of D5 dopamine receptor mRNA in rat brain. *Neurosci Lett* **145**(2): 209-212.

Medeiros R, Cabrini DA, Calixto JB (2001). The "in vivo" and "ex vivo" roles of cyclooxygenase-2, nuclear factor-kappaB and protein kinases pathways in the up-regulation of B1 receptor-mediated contraction of the rabbit aorta. *Regul Pept* **97**(2-3): 121-130.

Meltzer HY (2004). What's atypical about atypical antipsychotic drugs? *Curr Opin Pharmacol* **4**(1): 53-57.

Menke JG, Borkowski JA, Bierilo KK, MacNeil T, Derrick AW, Schneck KA, Ransom RW, Strader CD, Linemeyer DL, Hess JF (1994). Expression cloning of a human B1 bradykinin receptor. *J Biol Chem* **269**(34):21583-21586.

Merchenthaler I, Maderdrut JL, O'Harte F, Conlon JM (1992). *Peptides* **13**(4):815-29. Localization of neurokinin B in the central nervous system of the rat.

Meylan E, Tschöppe J (2008). IRAK2 takes its place in TLR signaling. *Nat Immunol* **9**(6): 581-582.

Michaud N, Couture R (2003). Cardiovascular and behavioural effects induced by naloxone-precipitated morphine withdrawal in rat: characterization with tachykinin antagonists. *Neuropeptides* **37**(6):345-354.

Milton AS, Wendlandt S (1970). A possible role for prostaglandin E1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol* **207**(2):76P-77P.

Minamino N, Masuda H, Kangawa K, Matsuo H (1984). Regional distribution of neuromedin K and neuromedin L in rat brain and spinal cord. *Biochem Biophys Res Commun* **124**(3): 731-738.

Minshall RD, Erdos EG, Vogel SM (1997a). Angiotensin I-converting enzyme inhibitors potentiate bradykinin's inotropic effects independently of blocking its inactivation. *Am J Cardiol* **80**(3A): 132A-136A.

Minshall RD, Tan F, Nakamura F, Rabito SF, Becker RP, Marcic B (1997b). Potentiation of the actions of bradykinin by angiotensin I-converting enzyme inhibitors. The role of expressed human bradykinin B2 receptors and angiotensin I-converting enzyme in CHO cells. *Circ Res* **81**(5): 848-856.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG (1998). Dopamine receptors: from structure to function. *Physiol Rev* **78**(1):189-225.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA (2005). Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Mol Psychiatry* **10**(1): 79-104.

Morand-Contant M, Anand-Srivastava, MB, Couture R (2010). Kinin B₁ receptor upregulation by angiotensin II and endothelin-1 in rat vascular smooth muscle cells: Receptors and mechanisms. *Am J Physiol*. September 10th 2010 (*in press*).

Mori T, Imamura T (1998). Inhibition of guinea pig skin allergic reactions by nonpeptide bradykinin B2 receptor antagonist FR173657. *Int Arch Allergy Immunol* **116**(4): 278-283.

Munoz CM, Cotecchia S, Leeb-Lundberg LM (1993). B2 kinin receptor-mediated internalization of bradykinin in DDT1 MF-2 smooth muscle cells is paralleled by sequestration of the occupied receptors. *Arch Biochem Biophys* **301**(2): 336-344.

Munoz CM, Leeb-Lundberg LM (1992). Receptor-mediated internalization of bradykinin. DDT1 MF-2 smooth muscle cells process internalized bradykinin via multiple degradative pathways. *J Biol Chem* **267**(1): 303-309.

Murone C, Paxinos G, McKinley MJ, Oldfield BJ, Muller-Esterl W, Mendelsohn FA (1997). Distribution of bradykinin B2 receptors in sheep brain and spinal cord visualized by in vitro autoradiography. *J Comp Neurol* **381**(2): 203-218.

Murone C, Perich RB, Schlawe I, Chai SY, Casley D, MacGregor DP (1996). Characterization and localization of bradykinin B2 receptors in the guinea-pig using a radioiodinated HOE140 analogue. *Eur J Pharmacol* **306**(1-3): 237-247.

Murtra P, Sheasby AM, Hunt SP, De Felipe C (2000). Rewarding effects of opiates are absent in mice lacking the receptor for substance P. *Nature* **405**(6783): 180-183.

Mussap CJ, Geraghty DP, Burcher E (1993). Tachykinin receptors: a radioligand binding perspective. *J Neurochem* **60**(6):1987-2009.

Nagaraja TN, Patel P, Gorski M, Gorevic PD, Patlak CS, Fenstermacher JD (2005). In normal rat, intraventricularly administered insulin-like growth factor-1 is rapidly cleared from CSF with limited distribution into brain. *Cerebrospinal Fluid Res* **2**: 5-8.

Nakajima Y, Nakajima S, Inoue M (1988). Pertussis toxin-insensitive G protein mediates substance P-induced inhibition of potassium channels in brain neurons. *Proc Natl Acad Sci USA* **85**(10):3643-3647.

Nagashima A, Takano Y, Tateishi K, Matsuoka Y, Hamaoka T, Kamiya H (1989). Central pressor actions of neurokinin B: increases in neurokinin B contents in discrete nuclei in spontaneously hypertensive rats. *Brain Res* **499**(1): 198-203.

Nakanishi S (1991). Mammalian tachykinin receptors. *Annu Rev Neurosci* **14**: 123-136.

Nakanishi S (1987). Substance P precursor and kininogen: their structures, gene organizations, and regulation. *Physiol Rev* **67**(4): 1117-1142.

Narumi S, Maki Y (1978). Stimulatory effects of substance P on neurite extension and cyclic AMP levels in cultured neuroblastoma cells. *J Neurochem* **30**(6):1321-1326.

Nawa H, Doteuchi M, Igano K, Inouye K, Nakanishi S (1984a). Substance K: a novel mammalian tachykinin that differs from substance P in its pharmacological profile. *Life Sci* **34**(12): 1153-1160.

Nawa H, Kotani H, Nakanishi S (1984b). Tissue-specific generation of two preprotachykinin mRNAs from one gene by alternative RNA splicing. *Nature* **312**(5996): 729-734.

Neves SR, Ram PT, Iyengar R (2002). "G protein pathways". *Science* **296** (5573): 1636-1639.

Nevin K, Zhuo H, Helke CJ (1994). Neurokinin A coexists with substance P and serotonin in ventral medullary spinally projecting neurons of the rat. *Peptides* **15**(6):1003-1011.

Ni A, Chai KX, Chao L, Chao J (1998a). Molecular cloning and expression of rat bradykinin B1 receptor. *Biochim Biophys Acta* **1442**(2-3): 177-185.

Ni A, Chao L, Chao J (1998b). Transcription factor nuclear factor kappaB regulates the inducible expression of the human B1 receptor gene in inflammation. *J Biol Chem* **273**(5): 2784-2791.

Nishimura K, Ward P, Erdos EG (1980). Kallikrein and renin in the membrane fractions of the rat kidney. *Hypertension* **2**(4): 538-545.

Noll G, Wenzel RR, Binggeli C, Corti C, Lüscher TF (1998). Role of sympathetic nervous system in hypertension and effects of cardiovascular drugs. *Eur Heart J* **19** Suppl F:F32-38.

O'Brien DP, White FJ (1987). Inhibition of non-dopamine cells in the ventral tegmental area by benzodiazepines: relationship to A10 dopamine cell activity. *Eur J Pharmacol* **142**(3): 343-354.

Oades RD, Halliday GM (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Res* **434**(2): 117-165.

Oates JA, Gillespie L, Udenfriends S, Sjoerdsma A (1960). Decarboxylase inhibition and blood pressure reduction by α -methyl-3, 4-dihydroxyd, l-phenylalanine. *Science* **131**: 1890-1891.

Ohkubo H, Nakanishi S (1991). Molecular characterization of the three tachykinin receptors. *Ann N Y Acad Sci* **632**:53-62.

Okamoto H, Greenbaum LM (1983). Isolation and structure of T-kinin. *Biochem Biophys Res Commun* **112**(2):701-708.

Olson VG, Nestler EJ (2007). Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. *Synapse* **61**(2):87-95.

Ongali B, Campos MM, Petcu M, Rodi D, Cloutier F, Chabot JG (2004). Expression of kinin B1 receptors in the spinal cord of streptozotocin-diabetic rat. *Neuroreport* **15**(16): 2463-2466.

Ongali B, Hellal F, Rodi D, Plotkine M, Marchand-Verrecchia C, Pruneau D (2006). Autoradiographic analysis of mouse brain kinin B1 and B2 receptors after closed head trauma and ability of Anatibant mesylate to cross the blood-brain barrier. *J Neurotrauma* **23**(5): 696-707.

Orawski AT, Simmons WH (1989). Degradation of bradykinin and its metabolites by rat brain synaptic membranes. *Peptides* **10**(5): 1063-1073.

Otsuka M, Yoshioka K (1993). Neurotransmitter functions of mammalian tachykinins. *Physiol Rev* **73**(2): 229-308.

Oury-Donat F, Carayon P, Thurneyssen O, Pailhon V, Emonds-Alt X, Soubrie P (1995). Functional characterization of the nonpeptide neurokinin3 (NK3) receptor antagonist, SR142801 on the human NK-3R expressed in Chinese hamster ovary cells. *J Pharmacol Exp Ther* **274**(1): 148-154.

Overton P, Elliott PJ, Hagan RM, Clark D (1992). Neurokinin agonists differentially affect A9 and A10 dopamine cells in the rat. *Eur J Pharmacol* **213**(1): 165-166.

Page NM, Bell NJ, Gardiner SM, Manyonda IT, Brayley KJ, Strange PG, Lowry PJ (2003). Characterization of the endokinins: human tachykinins with cardiovascular activity. *Proc Natl Acad Sci U S A* **100**:6245-6250.

Page NM (2004). Hemokinins and endokinins. *Cell Mol Life Sci* **61**(13): 1652-1663.

Page NM (2005). New challenges in the study of the mammalian tachykinins. *Peptides* **26**(8): 1356-1368.

Palkovits M, Young WS, 3rd, Kovacs K, Toth Z, Makara GB (1998). Alterations in corticotropin-releasing hormone gene expression of central amygdaloid neurons following long-term paraventricular lesions and adrenalectomy. *Neuroscience* **85**(1): 135-147.

Patacchini R, Lecci A, Holzer P, Maggi CA (2004). Newly discovered tachykinins raise new questions about their peripheral roles and the tachykinin nomenclature. *Trends Pharmacol Sci* **25**(1): 1-3.

Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; San Diego, CA: 1998.

Penny GR, Afsharpour S, Kitai ST (1986). Substance P-immunoreactive neurons in the neocortex of the rat: a subset of the glutamic acid decarboxylase-immunoreactive neurons. *Neurosci Lett* **65**(1): 53-59.

Perkins MN, Kelly D (1993). Induction of bradykinin B1 receptors in vivo in a model of ultra-violet irradiation-induced thermal hyperalgesia in the rat. *Br J Pharmacol* **110**(4): 1441-1444.

Perkins MN, Kelly D, Davis AJ (1995). Bradykinin B1 and B2 receptor mechanisms and cytokine-induced hyperalgesia in the rat. *Can J Physiol Pharmacol* **73**(7): 832-836.

Perry DC, Snyder SH (1984). Identification of bradykinin in mammalian brain. *J Neurochem* **43**(4): 1072-1080.

Pesquero JB, Lindsey CJ, Zeh K, Paiva AC, Ganten D, Bader M (1994). Molecular structure and expression of rat bradykinin B2 receptor gene. Evidence for alternative splicing. *J Biol Chem* **269**(43): 26920-26925.

Pesquero JB, Pesquero JL, Oliveira SM, Roscher AA, Metzger R, Ganter D, Bader M (1996). Molecular cloning and functional characterization of a mouse bradykinin B₁ receptor gene. *Biochem. Biophys. Res. Commun* **220**:219-225.

Petcu M, Dias JP, Ongali B, Thibault G, Neugebauer W, Couture R (2008). Role of kinin B1 and B2 receptors in a rat model of neuropathic pain. *Int Immunopharmacol* **8**(2): 188-196.

Petcu M, Ongali B, El Midaoui A, de Champlain J, Couture R (2005). Effects of alpha-lipoic acid on kinin B1 and B2 receptor binding sites in the spinal cord of chronically angiotensin-treated rats. *Peptides* **26**(8): 1331-1338.

Plummer AJ, Yonkman FF (1960). The influence of antihypertensive and hypertensive substances on vascular reactivity to catecholamines. *Am J Cardiol.* **5**:640-648.

Phagoo SB, Poole S, Leeb-Lundberg LM (1999). Autoregulation of bradykinin receptors: agonists in the presence of interleukin-1beta shift the repertoire of receptor subtypes from B2 to B1 in human lung fibroblasts. *Mol Pharmacol* **56**(2): 325-333.

Phagoo SB, Yaqoob M, Herrere-Martinez E, McIntyre P, Jones C, and Burgess GM (2000) Regulation of bradykinin receptor gene expression in human lung fibroblasts. *Eur J Pharmacol* **397**: 237-246.

Phillipson OT (1979). Afferent projections to the ventral tegmental area of Tsai and interfascicular nucleus: a horseradish peroxidase study in the rat. *J Comp Neurol* **187**(1): 117-143.

Picard P, Boucher S, Regoli D, Gitter BD, Howbert JJ, Couture R (1993). Use of non-peptide tachykinin receptor antagonists to substantiate the involvement of NK-1 and NK-2Rs in a spinal nociceptive reflex in the rat. *Eur J Pharmacol* **232**(2-3): 255-261.

Picard P, Regoli D, Couture R (1994). Cardiovascular and behavioural effects of centrally administered tachykinins in the rat: characterization of receptors with selective antagonists. *Br J Pharmacol* **112**(1): 240-249.

Pickel VM, Chan J, Nirenberg MJ (2002). Region-specific targeting of dopamine D2-receptors and somatodendritic vesicular monoamine transporter 2 (VMAT2) within ventral tegmental area subdivisions. *Synapse* **45**(2): 113-124.

Pierce RC, Kumaresan V (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev* **30**(2): 215-238.

Pinto FM, Almeida TA, Hernandez M, Devillier P, Advenier C, Candenas ML (2004). mRNA expression of tachykinins and tachykinin receptors in different human tissues. *Eur J Pharmacol* **494**(2-3): 233-239.

Pinto YM, Paul M, Ganten D (1998). Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovasc Res* **39**(1): 77-88.

Plante GE (1999). Angiotensin II receptor antagonists: enlarging therapeutic possibilities in arterial hypertension. *Can J Cardiol* **15 Suppl F**: 4F-6F.

Placenza FM, Fletcher PJ, Rotzinger S, Vaccarino FJ (2004). Infusion of the substance P analogue, DiMe-C7, into the ventral tegmental area induces reinstatement of cocaine-seeking behaviour in rats. *Psychopharmacology (Berl)* **177**(1-2):111-120.

Plesnila N, Schulz J, Stoffel M, Eriska J, Pruneau D, Baethmann A (2001). Role of bradykinin B2 receptors in the formation of vasogenic brain edema in rats. *J. Neurotrauma* **18**:1049 – 1058.

Polidori C, Saija A, Perfumi M, Costa G, De Caro G, Massi M (1989). Vasopressin release induced by intracranial injection of tachykinins is due to activation of central neurokinin-3 receptors. *Neurosci Lett* **103**: 320-325.

Porreca F, Vanderah TW, Guo W, Barth M, Dodey P, Peyrou V (2006). Antinociceptive pharmacology of N-[[4-(4,5-dihydro-1H-imidazol-2-yl)phenyl]methyl]-2-[2-[[[(4-methoxy-2,6-dimethylphenyl)sulfonyl]methylamino]ethoxy]-N-methylacetamide, fumarate (LF22-0542), a novel nonpeptidic bradykinin B1 receptor antagonist. *J Pharmacol Exp Ther* **318**(1): 195-205.

Prat A, Picard P, Couture R (1994). Cardiovascular and behavioural effects of centrally administered neuropeptide K in the rat: receptors characterization. *Br J Pharmacol* **112**: 250-256.

Prat A, Biernacki K, Pouly S, Nalbantoglu J, Couture R, Antel JP (2000). Kinin B1 receptor expression and function on human brain endothelial cells. *J Neuropathol Exp Neurol* **59**(10):896-906.

Preston Z, Richardson PJ, Pinnock RD, Lee K (2000). NK-3 receptors are expressed on mouse striatal gamma-aminobutyric acid-ergic interneurons and evoke [(3)H] gamma-aminobutyric acid release. *Neurosci Lett* **284**(1-2): 89-92.

Priestley JV, Kelly JS, Cuello AC (1979). Uptake of [3H]dopamine in periglomerular cells of the rat olfactory bulb: an autoradiographic study. *Brain Res* **165**(1): 149-155.

Privitera PJ (1992). Brain kallikrein-kinin system in arterial pressure regulation. *Agents Actions Suppl* **38** (Pt 3):39-46.

Privitera PJ, Beckstead RM, Yates P, Walgren R (2003). Autoradiographic localization of [125I-Tyr0]bradykinin binding sites in brains of Wistar-Kyoto and spontaneously hypertensive rats. *Cell Mol Neurobiol* **23**(4-5): 805-815.

Quintao NL, Passos GF, Medeiros R, Paszcuk AF, Motta FL, Pesquero JB (2008). Neuropathic pain-like behaviour after brachial plexus avulsion in mice: the relevance of kinin B1 and B2 receptors. *J Neurosci* **28**(11): 2856-2863.

Rachinsky M, Pruneau D, Artru AA, Kapuler V, Alonchin A, Smolanezki Y, Shapira Y (2001). The importance of kinin antagonist treatment timing in closed head trauma. *J Trauma* **51**:944-948.

Raidoo DM, Bhoola KD (1997). Kinin receptors on human neurones. *J Neuroimmunol* **77**(1): 39-44.

Raidoo DM, Ramsaroop R, Naidoo S, Bhoola KD (1996). Regional distribution of tissue kallikrein in the human brain. *Immunopharmacology* **32**(1-3): 39-47.

Ranaldi R, Wise RA (2001). Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. *J Neurosci* **21**(15): 5841-5846.

Rang HP, Dale MM and Ritter JM (2001). Other transmitters and modulators. In: *Pharmacology*, 4th edition. Edinburgh, UK: Harcourt Publishers Ltd 483–493.

Rao PJ, Bhattacharya SK (1988). Hyperthermic effect of centrally administered bradykinin in the rat: role of prostaglandins and serotonin. *Int J Hyperthermia* **4**(2): 183-189.

Rashid MH, Inoue M, Matsumoto M, Ueda H (2004). Switching of bradykinin-mediated nociception following partial sciatic nerve injury in mice. *J Pharmacol Exp Ther* **308**(3):1158-1164.

Regoli D (2000). Toward a new anti-inflammatory and analgesic agent. *Proc Natl Acad Sci U S A* **97**(14): 7676-7677.

Regoli D, Barabe J (1980). Pharmacology of bradykinin and related kinins. *Pharmacol Rev* **32**(1): 1-46.

Regoli D, Barabe J, Park WK (1977). Receptors for bradykinin in rabbit aortae. *Can J Physiol Pharmacol* **55**(4): 855-867.

Regoli D, Jukic D, Gobeil F, Rhaleb NE (1993). Receptors for bradykinin and related kinins: a critical analysis. *Can J Physiol Pharmacol* **71**(8): 556-567.

Regoli D, Marceau F, Barabe J (1978). De novo formation of vascular receptors for bradykinin. *Can J Physiol Pharmacol* **56**(4): 674-677.

Regoli D, Nguyen QT, Jukic D (1994). Neurokinin receptor subtypes characterized by biological assays. *Life Sci* **54**(26): 2035-2047.

Regoli D, Nsa Allogho S, Rizzi A, Gobeil FJ (1998). Bradykinin receptors and their antagonists. *Eur J Pharmacol* **348**(1): 1-10.

Regoli D, Pheng LH, Allogho SN, Nguyen-Le XK, Gobeil F (1996). Receptors for kinins: from classical pharmacology to molecular biology. *Immunopharmacology* **33**(1-3): 24-31.

Regoli D, Rhaleb NE, Tousignant C, Rouissi N, Nantel F, Jukic D (1991). New highly potent bradykinin B2 receptor antagonists. *Agents Actions* **34**(1-2): 138-141.

Regoli D, Rizzi A, Perron SI, Gobeil F Jr (2001). Classification of kinin receptors. *Biol Chem* **382**(1): 31-35.

Regoli D (2004). Pharmacology of *nitric oxide*: molecular mechanisms and therapeutic strategies. *Curr. Pharm. Des.* **10**:1667-1676.

Reid JL, Lewis PJ, Myers MG, Dollery CT (1974). Cardiovascular effects of intracerebroventricular d-, 1- and dl-propranolol in the conscious rabbit. *J Pharmac Exp Ther* **188**: 394-399.

Reid IA, Nolan PL, Wolf JA, Keil LC (1979). Suppression of vasopressin secretion by clonidine: effect of alpha-adrenoceptor antagonists. *Endocrinology* **104**(5):1403-1406.

Reid JL (1986). Alpha-adrenergic and blood pressure control. *The American Journal of Cardiology* **57**(9) E6-E12.

Reis DJ, Morrison S, Ruggiero DA (1988). The C1 area of the brainstem in tonic and reflex control of blood pressure. State of the art lecture. *Hypertension* **11**(2 Pt 2):I8-13.

Rhaleb NE, Telemaque S, Rouissi N, Dion S, Jukic D, Drapeau G (1991). Structure-activity studies of bradykinin and related peptides. B2-receptor antagonists. *Hypertension* **17**(1): 107-115.

Ribeiro-da-Silva A, Hökfelt T (2000). Neuroanatomical localisation of Substance P in the CNS and sensory neurons. *Neuropeptides* **34**(5):256-271.

Rodi D, Couture R, Ongali B, Simonato M (2005). Targeting kinin receptors for the treatment of neurological diseases. *Curr Pharm Des* **11**(10): 1313-1326.

Roccon A, Marchionni D, Nisato D (1996). Study of SR 142801, a new potent non-peptide NK-3R antagonist on cardiovascular responses in conscious guinea-pig. *Br J Pharmacol* **118** (5): 1095-102.

Rocha e Silva M, Beraldo WT, Rosenfield G (1949). Bradykinin, a hypotensive and smooth muscles stimulating factor released from plasma by snake venoms and by trypsin. *Am J Physiol* **156**: 261-273.

Rocha e Silva M (1949). Autofarmacologia e venenos animais. *Arq Inst Biol S Paulo* **19**:1-22.

Rocha e Silva M, Corrado AP, Ramos AO (1960). Potentiation of duration of the vasodilator effect of bradykinin by sympatholytic drugs and by reserpine. *J Pharmacol Exp Ther* **128**: 217-226.

Rockhold RW, Caldwell RW (1980). Cardiovascular effects following clonidine microinjection into the nucleus tractus solitarii of the rat. *Neuropharmacology* **19**(9):919-22.

Rodriguez AI, Pereira-Flores K, Hernandez-Salinas R, Boric MP, Velarde V (2006). High glucose increases B1-kinin receptor expression and signaling in endothelial cells. *Biochem Biophys Res Commun* **345**(2): 652-659.

Rodriguez JJ, Doherty MD, Pickel VM (2000). N-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area: subcellular distribution and colocalization with 5-hydroxytryptamine(2A) receptors. *J Neurosci Res* **60**(2): 202-211.

Rosén A, Brodin K, Eneroth P, Brodin E (1992). Short-term restraint stress and s.c. saline injection alter the tissue levels of substance P and cholecystokinin in the peri-aqueductal grey and limbic regions of rat brain. *Acta Physiol Scand.* **146**(3):341-8.

Roth RH, Allikmets L, Delgado JM (1969). Synthesis and release of noradrenaline and dopamine from discrete regions of monkey brain. *Arch Int Pharmacodyn Ther* **181**(2): 273-282.

Rovero P, Pestellini V, Patacchini R, Giuliani S, Santicioli P, Maggi CA (1989). A potent and selective agonist for NK-2 tachykinin receptor. *Peptides* **10**(3): 593-595.

Rupniak NM, Kramer MS (1999). Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK-1) antagonists. *Trends Pharmacol Sci* **20**(12): 485-490.

Rupniak NM, Boyce S, Williams AR, Cook G, Longmore J, Seabrook GR, Caesar M, Iversen SD, Hill RG (1993). Antinociceptive activity of NK1 receptor antagonists: non-specific effects of racemic RP67580. *Br J Pharmacol.* **110**(4):1607-13.

Russell VA, Sagvolden T, Johansen EB (2005). Animal models of attention-deficit hyperactivity disorder. *Behav Brain Funct* **1**: 1-17.

Sabourin CL, Danne MM, Buxton KL, Casillas RP, Schlager JJ (2002). Cytokine, chemokine, and matrix metalloproteinase response after sulfur mustard injury to weanling pig skin. *J Biochem Mol Toxicol* **16**(6): 263-272.

Saffroy M, Beaujouan JC, Torrens Y, Besseyre J, Bergstrom L, Glowinski J (1988). Localization of tachykinin binding sites (NK-1, NK2, NK3 ligands) in the rat brain. *Peptides* **9**(2): 227-241.

Saffroy M, Torrens Y, Glowinski J, Beaujouan JC (2003). Autoradiographic distribution of tachykinin NK2 binding sites in the rat brain: comparison with NK-1 and NK3 binding sites. *Neuroscience* **116**(3): 761-773.

Sakata M, Sei H, Toida K, Fujihara H, Urushihara R, Morita Y (2002). Mesolimbic dopaminergic system is involved in diurnal blood pressure regulation. *Brain Res* **928**(1-2):194-201.

Saklatvala J, Dean J, Finch A (1999). Protein kinase cascades in intracellular signalling by interleukin-I and tumour necrosis factor. *Biochem Soc Symp* **64**: 63-77.

Sales N, Martres MP, Bouthenet ML, Schwartz JC (1989). Ontogeny of dopaminergic D-2 receptors in the rat nervous system: characterization and detailed autoradiographic mapping with [125I]iodosulpride. *Neuroscience* **28**(3): 673-700.

Salvino JM, Seoane PR, Douty BD, Awad MM, Dolle RE, Houck WT (1993). Design of potent non-peptide competitive antagonists of the human bradykinin B2 receptor. *J Med Chem* **36**(17): 2583-2584.

Sarau HM, Griswold DE, Bush B, Potts W, Sandhu P, Lundberg D (2000). Nonpeptide tachykinin receptor antagonists. II. Pharmacological and pharmacokinetic profile of SB-222200, a central nervous system penetrant, potent and selective NK-3 receptor antagonist. *J Pharmacol Exp Ther* **295**(1): 373-381.

Sardi SP, Errasti AE, Rey-Ares V, Rogines-Velo MP, Rothlin RP (2000). Bradykinin B1 receptor in isolated human umbilical vein: an experimental

model of the in vitro up-regulation process. *Acta Pharmacol Sin* **21**(2): 105-110.

Sardi SP, Rey-Ares V, Pujol-Lereis VA, Serrano SA, Rothlin RP (2002). Further pharmacological evidence of nuclear factor-kappa B pathway involvement in bradykinin B1 receptor-sensitized responses in human umbilical vein. *J Pharmacol Exp Ther* **301**(3): 975-980.

Sawutz DG, Salvino JM, Dolle RE, Casiano F, Ward SJ, Houck WT, Faunce DM, Douty BD, Baizman E, Awad MM (1994). The nonpeptide WIN 64338 is a bradykinin B2 receptor antagonist. *Proc Natl Acad Sci USA* **91**(11):4693-4697.

Schanstra JP, Bataille E, Marin Castano ME, Barascud Y, Hirtz C, Pesquero JB, Pecher C, Gauthier F, Girolami JP, Bascands JL (1998). The B1-agonist [des-Arg10]-kallidin activates transcription factor NF-kappaB and induces homologous up-regulation of the bradykinin B1-receptor in cultured human lung fibroblasts. *J Clin Invest* **10**(1):2080-2091.

Sherman MS, Sekiguchi K, Nishizuka Y (1989). Modulation of ion channel activity: a key function of the protein kinase C enzyme family. *Pharmacological Reviews* **41**: 211-237.

Scicli AG, Forbes G, Nolly H, Dujovny M, Carretero OA (1984). Kallikrein-kinins in the central nervous system. *Clin Exp Hypertens A* **6**(10-11): 1731-1738.

Schmidlin F, Scherrer D, Landry Y, Gies JP (1998). Glucocorticoids inhibit the bradykinin B2 receptor increase induced by interleukin-1beta in human bronchial smooth muscle cells. *Eur J Pharmacol* **354**(1):R7-8.

Schmitt H, Fenard S (1973). Action of alpha-adrenergic drugs on sympathetic centres and their interactions with the central sympathoinhibitory effects of clonidine. *Arzneimittel-Forsch* **23**: 40-45.

Schreihofer AM, Guyenet PG (2000). Role of presympathetic C1 neurons in the sympatholytic and hypotensive effects of clonidine in rats. *Am J Physiol Regul Integr Comp Physiol* **279**(5):R1753-62.

Schwartz JC, Malfroy B, De La Baum S (1981). Biological inactivation of enkephalins and the role of enkephalin- dipeptidyl-carboxypeptidase ("enkephalinase") as neuropeptidase. *Life Sci* **29**:1715-1740.

Seabrook GR, Bowery BJ, Hill RG (1995). Pharmacology of tachykinin receptors on neurones in the ventral tegmental area of rat brain slices. *Eur J Pharmacol* **273**(1-2): 113-119.

Seeger, T.F, Bartlett, B, Coskran, TM, Culp, JM, James, LC, Krull, DL, Lanfear, J, Ryan, AM, Schmidt, CJ, Strick, CA, Varghese, AH, Williams, RD, Wylie, PG and Menniti, FS (2003) Immunohistochemical localization of PDE10A in the rat brain. *Brain Res.*, **985**:113-126.

Sergeyev V, Hokfelt T, Hurd Y (1999). Serotonin and substance P co-exist in dorsal raphe neurons of the human brain. *Neuroreport* **10**(18): 3967-3970.

Seroogy KB, Mehta A, Fallon JH (1987). Neurotensin and cholecystokinin coexistence within neurons of the ventral mesencephalon: projections to forebrain. *Exp Brain Res* **68**(2):277-289.

Seroogy K, Ceccatelli S, Schalling M, Hökfelt T, Frey P, Walsh J, Dockray G, Brown J, Buchan A, Goldstein M (1988). A subpopulation of dopaminergic neurons in rat ventral mesencephalon contains both neurotensin and cholecystokinin. *Brain Res* **455**(1):88-98.

Sesack SR, Pickel VM (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* **320**(2):145-160.

Severini C, Improta G, Falconieri-Erspamer G, Salvadori S, Erspamer V (2002). The tachykinin peptide family. *Pharmacol Rev* **54**(2): 285-322.

Seybold VS, Grkovic I, Portbury AL, Ding YQ, Shigemoto R, Mizuno N (1997). Relationship of NK-3R-immunoreactivity to subpopulations of neurons in rat spinal cord. *J Comp Neurol* **381**(4): 439-448.

Sharif NA, Whitting RL (1991). Identification of B2- bradykinin receptors in guinea pig brain regions, spinal cord and peripheral tissues. *Neurochem Int* **18**: 89-96.

Shen H (2008). Illustrated Pharmacology Memory Cards: *PharMnemonics*. Minireview. pp. 12.

Shigemoto R, Yokota Y, Tsuchida K, Nakanishi S (1990). Cloning and expression of a rat neuromedin K receptor cDNA. *J Biol Chem*. **265**(2):623–628.

Shults CW, Quirion R, Chronwall B, Chase TN, O'Donohue TL (1984). A comparison of the anatomical distribution of substance P and substance P receptors in the rat central nervous system. *Peptides* **5**(6): 1097-1128.

Siebeck M, Schorr M, Spannagl E, Lehner M, Fritz H, Cheronis JC (1998). B1 kinin receptor activity in pigs is associated with pre-existing infection. *Immunopharmacology* **40**(1): 49-55.

Siegel RA, Duker EM, Pahnke U, Wuttke W (1987). Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. *Neuroendocrinology* **46**(1): 75-81.

Simson JA, Dom R, Chao J, Woodley C, Chao L, Margolius HS (1985). Immunocytochemical localization of tissue kallikrein in brain ventricular epithelium and hypothalamic cell bodies. *J Histochem Cytochem* **33**(9): 951-953.

Skidgel RA (1992). Bradykinin-degrading enzymes: structure, function, distribution, and potential roles in cardiovascular pharmacology. *J Cardiovasc Pharmacol* **20**: S4-9.

Smith PW, Dawson LA (2008). Neurokinin 3 (NK3) receptor modulators for the treatment of psychiatric disorders. *Recent Pat CNS Drug Discov* **3**(1): 1-15.

Smith ME, Flynn FW (2000). Distribution of Fos-like immunoreactivity within the rat brain following intraventricular injection of the selective NK(3) receptor agonist senktide. *J Comp Neurol* **426**(3):413-428.

Smith PW, Wyman PA, Lovell P, Goodacre C, Serafinowska HT, Vong A (2009). New quinoline NK-3R antagonists with CNS activity. *Bioorg Med Chem Lett* **19**(3): 837-840.

Smith Y, Charara A, Parent A (1996). Synaptic innervation of midbrain dopaminergic neurons by glutamate-enriched terminals in the squirrel monkey. *J Comp Neurol* **364**(2): 231-253.

Snider RM, Constantine JW, Lowe JA, 3rd, Longo KP, Lebel WS, Woody HA (1991). A potent nonpeptide antagonist of the substance P (NK-1) receptor. *Science* **251**(4992): 435-437.

Snider RM, McKinney M, Forray C, Richelson E (1984). Neurotransmitter receptors mediate cyclic GMP formation by involvement of arachidonic acid and lipoxygenase. *Proc Natl Acad Sci USA* **81**: 3905-3909.

Snyman C, Naidoo Y, Narotam PK, Figueroa CD, Van Dellen JR, Bhoola KD (1994). Cellular localization of atrial natriuretic peptide and tissue kallikrein in the human hypothalamus. *Braz J Med Biol Res* **27**(8): 1877-1883.

Spitznagel H, Baulmann J, Blume A, Unger T, Culman J (2001). C-FOS expression in the rat brain in response to substance P and neurokinin B. *Brain Res* **916**(1-2): 11-21.

Spooren W, Riemer C, Meltzer H (2005). Opinion: NK-3R antagonists: the next generation of antipsychotics? *Nat Rev Drug Discov* **4**(12): 967-975.

Stahl SM (1999). Substance P and the neurokinins: novel peptide neurotransmitters in psychopharmacology. *J Clin Psychiatry* **60**(2): 77-78.

Steranka LR, Burch RM, Vavrek RJ, Stewart JM, Enna S (1988). Multiple bradykinin receptors: results of studies using a novel class of receptor antagonists. *Adv Exp Med Biol* **236**:111-27.

Starke K, Borowski E, Taube HD, Endo T (1977). Mechanism of action of antihypertensive agents. *Contrib Nephrol* **8**:151-161.

Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ (1998). Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J Neurosci* **18**(19): 8003-8015.

Steinberg R, Alonso R, Griebel G, Bert L, Jung M, Oury-Donat F (2001). Selective blockade of neurokinin-2 receptors produces antidepressant-like

effects associated with reduced corticotropin-releasing factor function. *J Pharmacol Exp Ther* **299**(2): 449-458.

Stitt JT. Prostaglandin E1 fever induced in rabbits. *J Physiol* **232**(1):163-179.

Stoessl AJ, Dourish CT, Iversen SD (1989). The NK-3 tachykinin agonist senktide elicits yawning and chewing mouth movements following subcutaneous administration in the rat. Evidence for cholinergic mediation. *Psychopharmacology (Berl)*. **95**(4):502-506.

Stoessl AJ, Dourish CT, Iversen SD (1990). Pharmacological characterization of the behavioural syndrome induced by the NK-3 tachykinin agonist senktide in rodents: evidence for mediation by endogenous 5-HT. *Brain Res* **517**(1-2):111-116.

Stoessl AJ, Szczutkowski E, Glenn B, Watson I (1991). Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. *Brain Res* **565**(2): 254-262.

Stoof JC, Kebabian JW (1984). Two dopamine receptors: biochemistry, physiology and pharmacology. *Life Sci* **35**(23): 2281-2296.

Straub JA, Akiyama A, Parmar P (1994). In vitro plasma metabolism of RMP-7. *Pharm Res* **11**(11): 1673-1676.

Stylianou E, Saklatvala J (1998). Interleukin-1. *Int J Biochem Cell Biol* **30**(10): 1075-1079.

Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedvall G (1998). D3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res* **779**(1-2): 58-74.

Svensson TH, Bunney BS, Aghajanian GK (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res* **92**(2):291-306.

Swanson CJ, Kalivas PW (2000). Regulation of locomotor activity by metabotropic glutamate receptors in the nucleus accumbens and ventral tegmental area. *J Pharmacol Exp Ther* **292**(1): 406-414.

Swanson LW (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* **9**(1-6): 321-353.

Takano Y, Nagashima A, Hagio T, Tateishi K, Kamiya H (1990). Role of central tachykinin peptides in cardiovascular regulation in rats. *Brain Res* **528**(2): 231-237.

Takano Y, Takeda Y, Yamada K, Kamiya H (1985). Substance K, a novel tachykinin injected bilaterally into the ventral tegmental area of rats increases behavioural response. *Life Sci* **37**(26): 2507-2514.

Takayama H, Ota Z, Ogawa N (1986). Effect of immobilization stress on neuropeptides and their receptors in rat central nervous system. *Regul Pept* **15**(3): 239-248.

Takahashi H, Bunag RD (1981). Centrally induced cardiovascular and sympathetic nerve responses to bradykinin in rats. *J Pharmacol Exp Ther* **216**:192-197.

Tamiya R, Hanada M, Kawai Y, Inagaki S, Takagi H (1990). Substance P afferents have synaptic contacts with dopaminergic neurons in the ventral tegmental area of the rat. *Neurosci Lett* **110**(1-2): 11-15.

Tateishi K, Matsuoka Y, Hamaoka T (1989). Establishment of highly specific radioimmunoassays for neurokinin A and neurokinin B and determination of tissue distribution of these peptides in rat central nervous system. *Regul Pept* **24**(3):245-257

Tatemoto K, Lundberg JM, Jornvall H, Mutt V (1985). Neuropeptide K: isolation, structure and biological activities of a novel brain tachykinin. *Biochem Biophys Res Commun* **128**(2): 947-953.

Tauscher J, Hussain T, Agid O, Verhoeff NP, Wilson AA, Houle S (2004). Equivalent occupancy of dopamine D1 and D2 receptors with clozapine: differentiation from other atypical antipsychotics. *Am J Psychiatry* **161**(9): 1620-1625.

Thomas GR, Thibodeaux H, Margolius HS, Privitera PJ (1984). Cerebrospinal fluid kinins and cardiovascular function. Effects of cerebroventricular melittin. *Hypertension* **6**(2 Pt 2):I46-50.

Tschöpe C, Picard P, Culman J, Prat A, Itoi K, Regoli D (1992). Use of selective antagonists to dissociate the central cardiovascular and behavioural effects of tachykinins on NK-1 and NK-2Rs in the rat. *Br J Pharmacol* **107**(3): 750-755.

Tschöpe C, Heringer-Walther S, Koch M, Spillmann F, Wendorf M, Hauke D, Bader M, Schultheiss HP, Walther T (2000). Myocardial bradykinin B2-receptor expression at different time points after induction of myocardial infarction. *J Hypertens* **18**(2):223-228.

Tully T, Bourtchouladze R, Scott R, Tallman J (2003). Targeting the CREB pathway for memory enhancers *Nat Rev Drug Discov* **2**:267-277.

Unger T, Carolus S, Demmert G, Ganten D, Lang RE, Maser-Gluth C (1988). Substance P induces a cardiovascular defense reaction in the rat: pharmacological characterization. *Circ Res* **63**(4): 812-820.

Unger T, Kaufmann-Buhler I, Scholkens B, Ganten D (1981). Brain converting enzyme inhibition: a possible mechanism for the antihypertensive action of captopril in spontaneously hypertensive rats. *Eur J Pharmacol* **70**(4): 467-478.

Vaidya RA, Vaidya AB, Van Woert MH, Kass NG (1970). *Metabolism: Clinical and Experimental* **19**: 1069.

Van Bockstaele EJ, Cestari DM, Pickel VM (1994). Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: potential sites for modulation of mesolimbic dopamine neurons. *Brain Res* **647**(2): 307-322.

Van Bockstaele EJ, Pickel VM (1995). GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res* **682**(1-2):215-221.

van den Buuse M, Versteeg DH, de Jong W (1986). Brain dopamine depletion by lesions in the substantia nigra attenuates the development of hypertension in the spontaneously hypertensive rat. *Brain Res* **368**(1):69-78.

van den Buuse M, Linthorst AC, Versteeg DH, de Jong W (1991). Role of brain dopamine systems in the development of hypertension in the spontaneously hypertensive rats. *Clin Exp Hypertens A* **13**:653-659.

van den Buuse M, Morton SJ, Cornish JL, Head GA (1996). Prolonged central effects of quinpirole on cardiovascular regulation. *J Pharmacol Exp Ther* **277**(1):473-83.

van Den Buuse M (1997). Pressor responses to brain dopaminergic stimulation. *Clin. Exp. Pharmacol. Physiol* **24**(9-10): 764-769.

van Den Buuse M, Catanzariti R (2000). Stimulation of the ventral tegmental area enhances the effect of vasopressin on blood pressure in conscious rats. *Br J Pharmacol* **129**: 29-36.

van Zwieten PA (1995). Central imidazoline receptors as a target for centrally acting antihypertensive drugs. *Pharm World Sci* **17**(6):186-190.

van Zwieten (2002). Cardiovascular drug therapy 1971 - 2002 and beyond. *Netherlands Heart Journal* **10**(12):534-538.

Vaughan CE, van den Buuse M, Roland BL (1999). Brain dopamine D₂ receptor mRNA levels are elevated in young spontaneously hypertensive rats. *Neuroscience Res* **34**(4):199-205.

Vavrek RJ, Stewart JM (1985). Competitive antagonists of bradykinin. *Peptides* **6**(2): 161-164.

Walker K, Dray A, Perkins M (1996a). Development of hyperthermia and hyperalgesia following intracerebroventricular administration of endotoxin in the rat: effect of kinin B₁ and B₂ receptor antagonists. *Immunopharmacology* **33**(1-3): 264-269.

Walker K, Dray A, Perkins M (1996b). Development of hyperthermia following intracerebroventricular administration of endotoxin in the rat: effect of kinin B₁ and B₂ receptor antagonists. *Br J Pharmacol* **117**(4): 684-688.

Wang P, Izotova L, Mariano TM, Donnelly RJ, Pestka S (1994). Construction and activity of phosphorylatable human interferon-alpha B₂ and interferon-alpha A/D. *J Interferon Res* **14**(1): 41-46.

Wang ZY, Hakanson R (1992). (+/-)-CP-96,345, a selective tachykinin NK-1 receptor antagonist, has non-specific actions on neurotransmission. *Br J Pharmacol* **107**(3): 762-765.

Wallace DM, Magnuson DJ, Gray TS (1992). Organization of amygdaloid projections to brainstem dopaminergic, noradrenergic, and adrenergic cell groups in the rat. *Brain Res Bull* **28**:447-454.

Warden MK, Young WS (1988). 3rd Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *J Comp Neurol* **272**(1): 90-113.

Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P (1991). D1 and D2 dopamine receptor mRNA in rat brain. *Proc Natl Acad Sci U S A* **88**(5): 1859-1863.

Werle E, Götze W, Kappler A (1937): Über die Wirkung des Kallikreins auf den isolierten Darm und über eine neue darmkontrahierende Substanz. *Biochem Z* **289**:217 - 233.

Werle E, Trautschold I, Leysath (1961). Isolierung und Struktur des Kallidins. *Hoppe-Seyl Z* **326**: 174-176.

Whalley ET, Fritz H, Geiger R (1983). Kinin receptors and angiotensin converting enzyme in rabbits basilar arteries. *Naunyn Schmiedebergs Arch Pharmacol* **324**(4): 296-301.

Williams TA, Barnes K, Kenny AJ, Turner AJ, Hooper NM (1992). A comparison of the zinc contents and substrate specificities of the endothelial and testicular forms of porcine angiotensin converting enzyme and the preparation of isoenzyme-specific antisera. *Biochem J* **288**: 875-881.

Wines DR, Brady JM, Pritchett DB, Roberts JL, MacDonald, RJ (1989). Ôrgization and expression of the rat kallikrein gene fhly. *J Bioch Chem* **264**: 7653-7662.

Wirth K, Breipohl G, Stechl J, Knolle J, Henke S, Scholkens B (1991). DesArg9-D-Arg[Hyp3,Thi5,D-Tic7,Oic8]bradykinin (desArg10-[Hoe140]) is a potent bradykinin B1 receptor antagonist. *Eur J Pharmacol* **205**(2): 217-218.

Wormser U, Laufer R, Hart Y, Chorev M, Gilon C, Selinger Z (1986). Highly selective agonists for substance P receptor subtypes. *EMBO J.* **5**(11):2805-2808.

Wotherspoon G, Winter J (2000). Bradykinin B1 receptor is constitutively expressed in the rat sensory nervous system. *Neurosci Lett* **294**(3): 175-178.

Wu J, Akaike T, Hayashida K, Miyamoto Y, Nakagawa T, Miyakawa K (2002). Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. *Int J Cancer* **98**(1): 29-35.

Yamashita K, Koide Y, Aiyoshi Y (1983). Effects of substance P on thyroidal cyclic AMP levels and thyroid hormone release from canine thyroid slices. *Life Sci* **32**(18):2163-2166.

Yamashita N, Hayashi A, Baba J, Sawa A (1997). Rolipram, a phosphodiesterase-4-selective inhibitor, promotes the survival of cultured rat dopaminergic neurons. *Jpn J Pharmacol.* **75**(2):155-159.

Yan TC, Hunt SP, Stanford SC (2009). Behavioural and neurochemical abnormalities in mice lacking functional tachykinin-1 (NK1) receptors: a model of attention deficit hyperactivity disorder. *Neuropharmacology* **57**(7-8):627-635.

Yang HY, Neff NH (1972). Distribution and properties of angiotensin converting enzyme of rat brain. *J Neurochem* **19**(10): 2443-2450.

Yang X, Polgar P. Genomic structure of the human bradykinin B1 receptor gene and preliminary characterization of its regulatory regions. *Biochem Biophys Res Commun* **222**(3):718-725.

Yang J, Bardes ES, Moore JD, Brennan J, Powers MA, Kornbluth S (1998). Control of cyclin B1 localization through regulated binding of the nuclear export factor CRM1. *Genes Dev* **12**(14): 2131-2143.

Yano K, Higashida H, Hattori H, Nozawa Y (1985). Bradykinin-induced transient accumulation of inositol triphosphate in neuron-like cell line NG108-15 cells. *FEBS Lett* **181**: 403-406.

Yokota Y, Sasai Y, Tanaka K, Fujiwara T, Tsuchida K, Shigemoto R (1989). Molecular characterization of a functional cDNA for rat substance P receptor. *J Biol Chem* **264**(30): 17649-17652.

Zerari F, Fischer J, Sagot MA, Frobert Y, Couraud JY, Conrath M (1998). Substance P receptor immunodetection in the spinal cord: comparative use

of direct anti-receptor antibody and anti-complementary peptide antibody. *Brain Res Bull* **46**(3): 263-268.

Zhang Y, Lu L, Furlonger C, Wu GE, Paige CJ (2000). Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis. *Nat Immunol* **1**(5): 392-397.

Zhang Y, Brovkovich V, Brovkovich S, Tan F, Lee BS, Sharma T, Skidgel RA (2007). Dynamic receptor-dependent activation of inducible nitric-oxide synthase by ERK-mediated phosphorylation of Ser745. *J Biol Chem* **282**(44):32453-32461.

Zheng F, Johnson SW (2003). Metabotropic glutamate and muscarinic cholinergic receptor-mediated preferential inhibition of N-methyl-D-aspartate component of transmissions in rat ventral tegmental area. *Neuroscience* **116**(4): 1013-1020.

Zhong Y, Wu CF (1991). Altered synaptic plasticity in *Drosophila* memory mutants with a defective cyclic AMP cascade. *Science* **251**: 198-201.

Zuzack JS, Burkard MR, Cuadrado DK, Greer RA, Selig WM, Whalley ET (1996). Evidence of a bradykinin B1 receptor in human ileum: pharmacological comparison to the rabbit aorta B1 receptor. *J Pharmacol Exp Ther* **277**(3): 1337-1343.